STUDIES ON OVARIAN MATURATION AND LARVAL DEVELOPMENT OF *PENAEUS MERGUIENSIS DE* MAN IN CAPTIVITY

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Dedicated

To

My Parents and Sisters

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सारांश

हाल के वर्षों में संसार के खाद्य बाज़ारों में झींगों केलिए हुई मांग तथा प्रग्रहण मात्स्यिकी में हुई अनिश्चितता झींगा जल कृषि के क्षेत्र में तीव्र भौगोलिक प्रयास उठाए जाने केलिए सहायक निकली. *पेनिअस मेरगुएन्सिस* डी मैन जो बनाना ष्रिंप नाम से लोकप्रिय है, परीक्षण के लिए उपयुक्त और उच्च शक्यता वाला एक प्रमुख वर्ग है. इस लिए वर्तमान अध्ययन के लिए इसे चुन लिया गया. इन झींगो के पुनरुत्पादकीय एवं डिभंकीय विकास के प्रमुख पहलुओं पर जांच की गई. नेत्रवृंत अपक्षरण दुनिया व्यापक स्पुटनशालाओं में झींगों के प्रेरित परिपक्वन केलिए उपयुक्त एक सामान्य तरीका है. अतः वर्तमान अध्ययन में विभिन्न वातावरणीय स्थितियों में जीने वाले विभिन्न आकार वाले प्रौढ़ मादा झींगों में प्रेरित परिपक्वन के लिए नेत्रवृंत अपक्षरण किया गया. अपक्षरण किए गए झींगों को पश्चजल वातावरण की अपेक्षा समुद्री वातावरण में रखे जाने पर सफल परिणाम निकला और छोटे आकार वाले झींगों में समान परीक्षण करने पर कोई असर नहीं हुआ. वर्ष में सर्दी के महीनों में अपक्षरण किए गए मादा झींगों में अंडाशय का परिपक्वन मंद गति में दिखाया पडा. प्रमस्तिष्क और वक्षीय गुच्छिका सार और 5-हाइड्रोक्सीट्रिप्टमिन जैसे बाह्य पदार्थों के प्रयोग से गोनाडो सोमाटिक इन्डेक्स में उल्लेखनीय वृद्धी हुई लेकिन 17-a हाइड्रोक्सीप्रोजेस्टरोन लगाए गए झींगों में कोई परिवर्तन नहीं पाया गया. लवणता एवं तापमान के विभिन्न संयोजनों में *पी. मेरगुएन्सिस* के डिंभकों का पालन करने पर देखा गया कि 33°c और 35‰ में अन्य तापमान - लवणता संयोजनों की अपेक्षा नोप्लियस से पश्च डिंभक अवस्था तक का विकास तेज़ी से संपन्न हुआ. पश्च डिंभक अवस्थाओं में अधिकतम अतिजीविता एवं बढ़ती 15‰ देखी गई. प्रग्रहण स्थिति में , शीघ्र परिपक्वन, तीव्र बढ़ती दर और लवणता और तापमान के संयोजन में डिंभकों की व्यापक अतिजीविता दिखाने में *पी. मेरगुएन्सिस* की क्षमता जलकृषि में परीक्षण करने के लिए इस जाति की साध्यता की ओर इशारा करती है.

ABSTRACT

The upsurge in recent years in the world food market and uncertainities of capture fisheries has helped in the intensification of global effort on shrimp aquaculture. *Penaeus merguiensis* de Man, popularly known as Banana shrimp, is an important candidate species with high potential for farming and hence was selected for the present study. The fundamental aspects of its reproductive physiology and larval development were investigated. Eyestalk ablation is the most commonly employed method to induce maturation of shrimps in hatcheries worldwide. Hence in the present study, eyestalk ablations were carried out on adult females of different sizes under varying environmental conditions to induce maturation. Successful results were obtained when ablated females were maintained in marine conditions compared to brackishwater environment, while no effect was visible when females of smaller sizes were subjected to similar treatments. Females ablated during the colder months of the year had a delayed response of ovarian maturation. Administration of exogenous substances like cerebral and thoracic ganglionic extracts and 5-hydroxytryptamine resulted in a significant increase in GSI, while the increase in GSI was not statistically significant when shrimps were administered 17-α hydroxyprogesterone. Larval rearing of *P. merguiensis* at different salinity and temperature combinations showed that the development during nauplius to postlarval stages was faster at 33^{0}C and 35‰compared to other tested salinity and temperature combinations. During the postlarval stages maximum survival and growth was observed at 15‰. The ability of *P. merguiensis* to reach maturation in captive environment, together with the fast growth rate and tolerance of larvae to a wide range of salinity and temperature combinations in captivity, indicates *P. merguiensis* to be a promising candidate species for aquaculture.

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PREFACE

Shrimps, the nutrient rich biomorph, have been the most preferred decapod crustacean and assume supremacy in the world seafood market. The universal acceptability and demand for shrimps as much sought after food item is the result of their unique flavour, texture and versatility. Even though shrimps constitute a lower volume than the freshwater fishes such as tilapia and carp in production, they attract a high price making them a significant component in value terms. The lure of shrimps has resulted in the over-exploitation of the resource in the inshore waters thereby causing concern over its conservation. Aquaculture of shrimps, the best possible alternative, has been gaining momentum and has emerged as a major frontier of shrimp production in the developing countries both for domestic consumption and export. The rapid growth over the past decade has kept the global volume of shrimp production expanding despite the levelling of marine catches. World exports of shrimps constituted 9,41,814 tonnes in 1997 (FAO, 1997). In view of the limited possibilities for increasing world fish production from wild in the future, it is felt imperative to expand aquaculture.

Aquaculture of shrimps has been almost synonymous with culture of the Tiger shrimp *Penaeus monodon,* which is presently the prime species for culture. But recently other species are being considered for diversification of aquaculture industry. *Penaeus merguiensis* de Man 1888, popularly known as Banana shrimp, being of commercial value, can be an alternative or rotational species with *Penaeus monodon* at least in certain parts of the Indian coast. It forms 2-3% of the total shrimp catch in North Karnataka Coast. *Penaeus merguiensis* with its substantial reproductive capability, fast growth rate and wide range of salinity tolerance appears to be a promising candidate species for aquaculture. Its ability to attain maturity in captive environment, ease in breeding and producing postlarvae and low protein dietary requirement are all the advantages of this species for aquaculture.

Controlled breeding of penaeid shrimps under captivity became a necessity as the demand for quality shrimp seeds (postlarvae) increased tremendously, due to large-scale expansion of shrimp farming activities all over the world. This was made possible by the pioneering work of Panouse (1943) on the endocrine regulation of reproduction in female prawn *Leander serratus*. In view of its application in aquaculture, significant advances have been made in endocrinology of crustacean in subsequent years. In spite of these works, knowledge on reproductive biology are still in its infancy and not many studies have been carried out on Indian *Penaeus merguiensis*, which is one of the best suited species for seed production in hatcheries. In this context, the present work is an effort made to study some aspects of the reproductive physiology and larval development of *Penaeus merguiensis*. The major objectives of the study were:

1. To study the changes in ovaries of *P. merguiensis* at different stages of maturity by means of histological and electrophoretic analysis.

2. To induce ovarian development and spawning of *P. merguiensis* in captivity by eyestalk ablation and injection of different exogenous substances.

3. To study the larval development of *P. merguiensis* under different environmental conditions.

The thesis starts with a general introduction on the subject under study, reviews similar works done by others and also highlights the importance of the present work. This is followed by the chapters covering various aspects of reproductive biology and larval development experiments carried out in the present investigation. Each chapter is with an introduction highlighting the importance of the aspect of the study and with a comprehensive review of literature on the subject, which is followed by the materials and methods adopted in the study. The results obtained on various aspects are described with suitable illustrations together with statistical analyses. Each chapter is discussed elaborately bringing in the significant findings in the present investigation.

Chapter I provides description on the morphology of the female reproductive organs and the events leading to maturation of ovary. The female maturation process was assessed based on gross morphological observations of the gonads and estimation of the Gonado-Somatic Index (GSI) at different stages of maturity. The process of oogenesis was also studied through histological methods. Based on the structural changes observed in the cytoplasm and the nucleus of oocytes and the yolk accumulation in a graded manner, the process of oogenesis has been classified into 5 stages *viz.* Previtellogenic (Stage I), Early vitellogenic (Stage II), Late vitellogenic (Stage III), Mature (Stage IV) and Spent (Stage V).

Chapter II deals with the characterisation of vitellin and vitellogenin, which includes electrophoretic studies on the changes in female haemolymph and ovarian profile observed with the advancement in maturation.

Chapter III deals with the induced maturation experiments conducted under different environmental conditions of nature and their impact on gonadal maturation. Maturation was induced by eyestalk ablation, injections of ganglionic extracts, synthetic hormone and neurotransmitter.

Chapter IV briefly describes the larval stages of *P. merguiensis* and also presents the results of larval rearing experiments carried under different environmental conditions.

A summary at the end highlights and enumerates the significant findings of the present study, followed by a list of literature consulted under references.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Despite fluctuations in supply and demand caused by the changing state of fisheries resources, the economic climate and environmental conditions, fisheries remain very important as a source of food, employment and revenue in many countries. The increasing demand for protein rich food from aquatic sources has resulted in the worldwide expansion of aquaculture. Reported global capture fisheries and aquaculture production for 1997 was 122 million tonnes while in 1998 it was only 117 million tonnes. The preliminary estimates for 1999 is about 125 million tonnes and this increase in production is mainly due to aquaculture as capture fisheries production remained relatively stable (FAO, 1999).

The continuing rise in production from aquaculture is paralleled by a diversification of the sector, with more and more species entering into culture systems. Crustacea constitutes a class, which includes species of biological interest and high commercial values. Shrimps, among the crustaceans, are the leading commodity in world market accounting for about 20% in total value of internationally traded fishery products. Despite its relative newness to aquaculture, close to a third of world shrimp landing is now made of farmed shrimps.

Penaeus merguiensis de Man, 1888 (Plate 1), popularly known as Banana shrimp, forms 5. 5% of the total world shrimp production (Yap, 1999). It occurs throughout the Asian and Australian tropical and subtropical waters from 67^0E to 166^0E and from 25^0N to 29^0S. It forms a minor fishery along the Karwar Coast (Latitude 14^0 48'N and Longitude 74^0 6' E) of Karnataka State (Figure1). Although production of *P. merguiensis* is not as large as that of other species of the genus *Penaeus* in Karwar, nevertheless, it represents an important regional fishing activity and assumes importance mainly due to its large size and export value in North Karnataka (1 - 2% in capture fishery). Therefore, it was felt necessary to

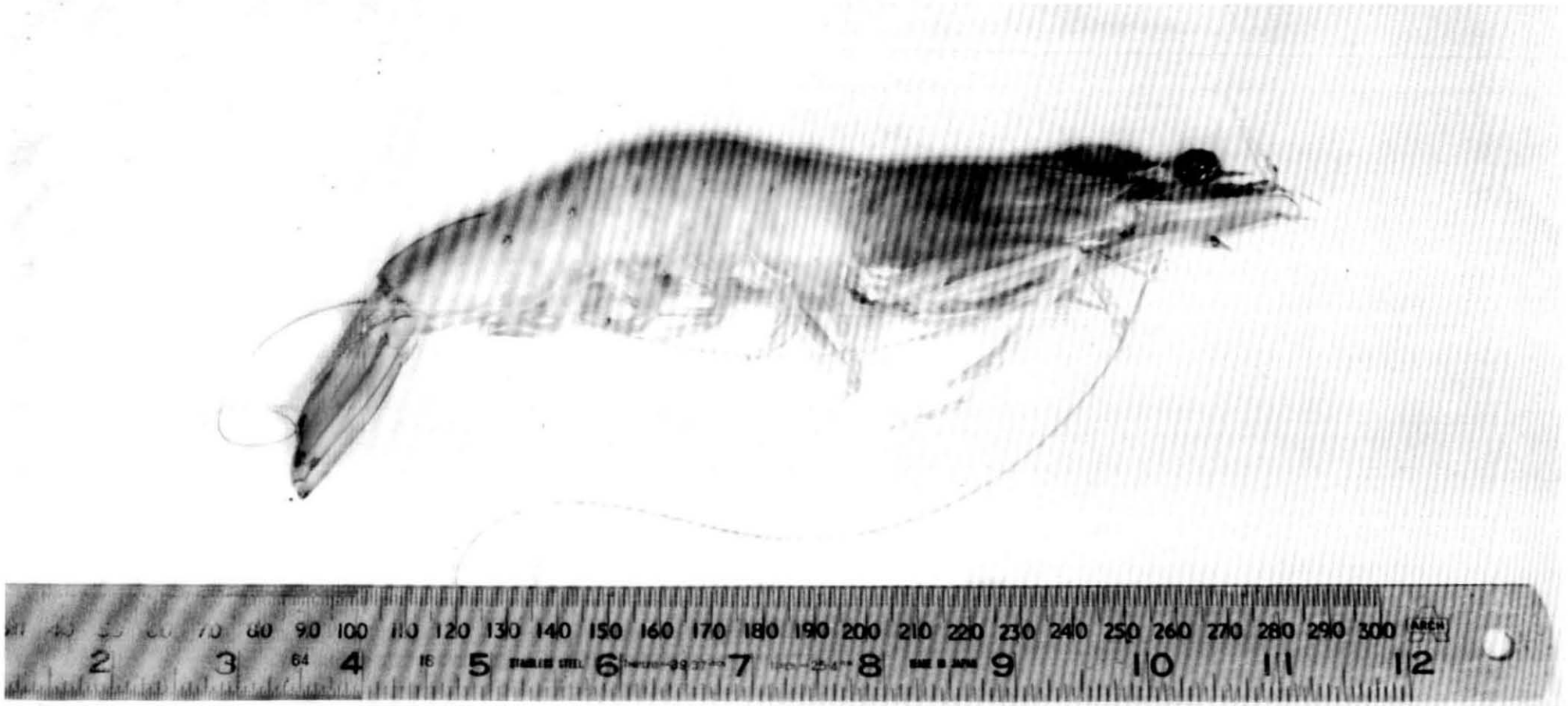
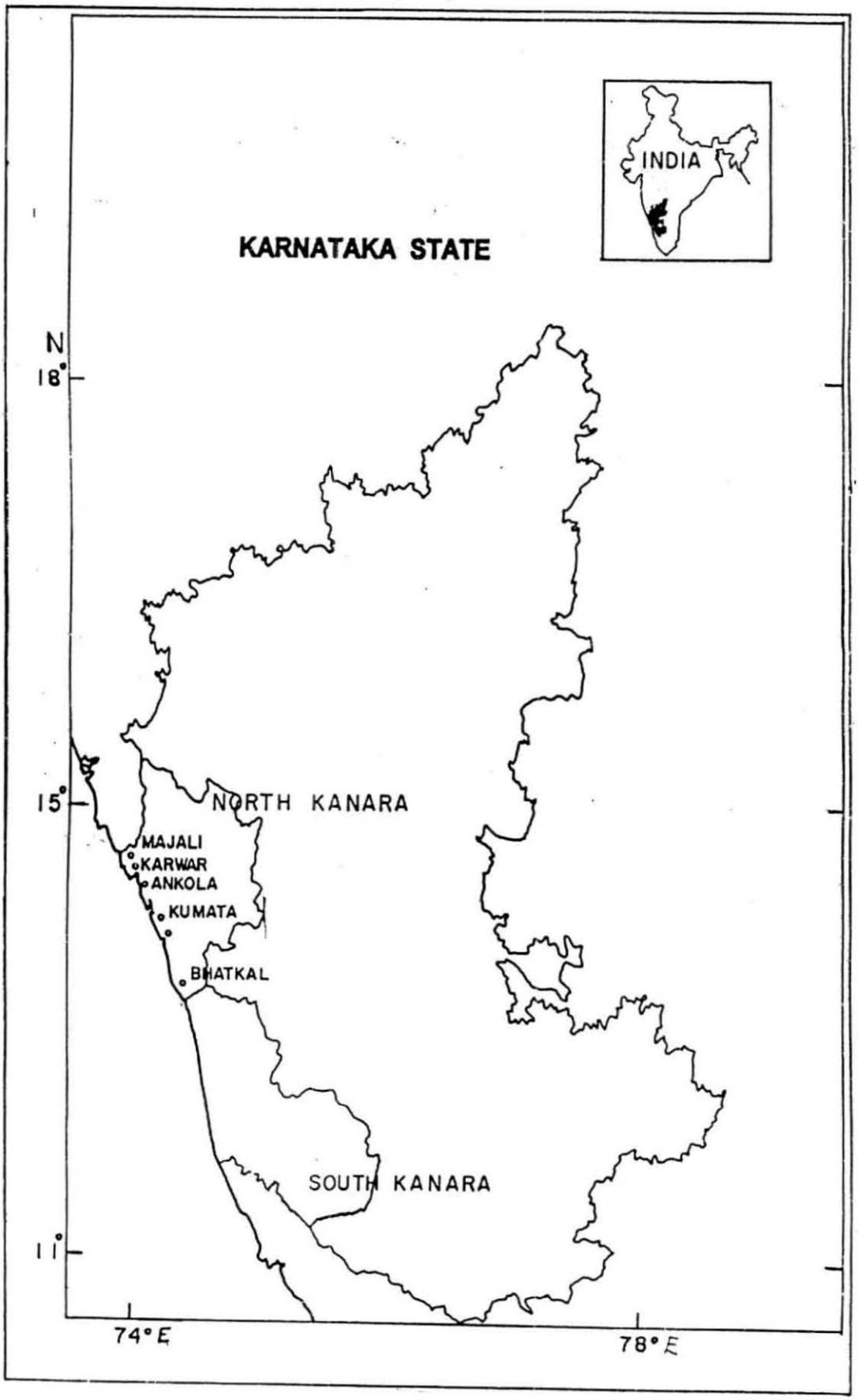

90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300
2 3 4 5 6 7 8 9 10 11 12

FIGURE 1. Map showing the North and South Kanara districts in Karnataka State along with sampling stations



acquire more and adequate information on various aspects of its reproductive physiology through the present investigation.

Reproduction in crustaceans requires a thorough knowledge of the morphological and physiological changes of the gonad. An understanding of the reproductive biology of the species is an essential prerequisite for stock assessment of wild population and for culture in confined habitats. The knowledge of inherent reproductive patterns of wild shrimp populations is also essential in predicting the expected performance of hatchery broodstock. To date there is a dearth of information in specific events during shrimp maturation. Definition of the developmental stages of the gonads and determination of practical gonadal indices represent a starting point for the study of the reproductive activities of the penaeid species. Gross morphological description together with the cytological changes associated with ovarian maturation in penaeid shrimps such as *P. japonicus* (Hudinaga, 1942; Yano, 1988), *P. duorarum* (Cummings, 1961), *P. merguiensis* (Tuma, 1967), P. *aztecus* and *P. setiferus* (Duronslet *et al.*, 1975), Sicyonia *ingentia* (Anderson *et al.*, 1984), *Parapenaeus longirostris* (Tom *et al.*, 1987), *P. indicus* (Mohamed, 1989), *P. monodon* (Joseph, 1996), *P. kerathurus* (Medidna *et al.*, 1996), *P. brasiliensis* (Sandoval Quintero and Gracia, 1998) and *Metapenaeopsis dalei* (Sakaji *et al.*, 2000) have been reported based on light microscopic as well as electron microscopic studies.

Protein level is known to vary in the ovaries and haemolymph during vitellogenesis. Vitellogenesis is the most important aspect of female reproductive cycle and many authors have determined the electrophoretic behaviour of vitellin and vitellogenin during egg formation (Adiyodi, 1968; Adiyodi and Adiyodi, 1970; Vazquez Boucard *et al.*, 1986; Tom *et al.*, 1987; Quinitio *et al.*, 1989, 1990; Shafir *et al.*, 1992; Chang *et al.*, 1993a, b; 1994, 1996; Sagi *et al.*, 1995; Lee *et al.*, 1997; Qiu *et al.*, 1997; Jasmani *et al.*, 2000; Kawazoe *et al.*, 2000). In order to understand the changes associated with maturation and breeding of shrimps, it is necessary to understand these alterations, which would provide an insight into

the factors affecting the normal growth and thus, the successful culture of the species.

Successful domestication of the candidate species is the footstep of any economically viable culture process and thus the evolution of modern shrimp culture requires the captive reproduction of the candidate species. However, lack of information on the basic physiological events that are taking place in the gonads as well as in related organs during different phases of gonadal maturation hampers shrimp aquaculture. Thus, control of reproductive maturation is a major problem for the development of commercial aquaculture programmes for penaeid shrimps. Reproduction, in crustaceans has been hypothesised to be controlled by dual endocrine factors - a gonad-inhibiting hormone (GIH) from the X organ-sinus gland complex and a gonad-stimulating hormone (GSH) secreted by the brain/ thoracic ganglion of maturing females, the actions of which are antagonistic to each other (Adiyodi and Adiyodi, 1970). In the majority of malacostracan crustaceans, eyestalk is the pivotal organ for housing various neuropeptides responsible in regulating reproduction in a number of crustacean species. Captive maturation has been achieved on a consistent, dependable basis for only a few species of penaeid shrimps and thus hatcheries continue to be heavily dependent on wild broodstock.

Various investigations are underway to develop techniques, which may lead to captive maturation. A proposed solution is endocrinological manipulation of captive broodstock. Endocrine manipulation to induce gonadal maturation has so far been synonymous with unilateral eyestalk ablation and has a far-reaching impact on crustacean aquaculture. This technique, which reduces the titre of GIH in females causing accelerated ovarian growth, has been extensively used by many with varying degrees of success (Arnstein and Beard, 1975; Alikunhi *et al.*, 1975; Aquacop, 1975; Lumare, 1979; Emmerson, 1980; Muthu and Laxminarayana, 1982; Primavera, 1982; Chamberlain and Gervais, 1984; Charniaux – Cotton and Payen, 1988; Meusy and Payen, 1988; Makinouchi and

Hirata, 1995; Ramos *et al.*, 1995; Jetani *et al.*, 1996; Joseph, 1996; Lumare *et al.*, 1996; Murugesan *et al.*, 1998; Palacios *et al.*, 1999).

Eyestalk ablation has the paradoxical effect of both stimulating the reproductive system while at the same time placing the females under additional stress. It is also associated with problems such as deterioration in spawn quality and quantity over time (Emmerson, 1980; Primavera, 1985) and manifesting conflicting results on spawn size, hatching success and other variables (Browdy, 1992). However, it has the beneficial effect of synchronising spawning and is hence of considerable importance in planning the production of postlarval cycles. In order to try dispose of a rapid and effective alternative to the existing technique (eyestalk ablation) other alternatives like implantation of ganglionic extracts (thoracic and cerebral ganglion) prepared from maturing females has been usually employed to trigger and control secondary vitellogenesis in penaeid shrimps (Otsu, 1960; Oyama, 1968; Hinsch and Bennett, 1979; Nagabhushanam and Kulkarni, 1982; Eastman-Reks and Fingerman, 1984; Takayanagi *et al.*, 1986; Kulkarni *et al.*, 1991; Yano, 1992; Yano and Wyban, 1992; Mohamed and Diwan, 1994; Joseph, 1996).

Crustaceans have the ability to synthesize vertebrate type steroid hormones. Although the mode of vertebrate hormone action on crustacean egg production is not properly understood, there appears to be a tremendous application potential in using these hormones to stimulate ovarian maturation (Kulkarni *et al.*, 1979; Yano, 1985; Yano, 1987; Chan and Lim, 1988; Joseph, 1996). The role of neurotransmitters in regulating gonadal maturation by inducing the release of neurohormones that regulate the reproductive activities in both vertebrates (Crim *et al.*, 1984) and invertebrates (Fingerman, 1987) has been recently understood. This aspect has also been tried on various crustaceans (Richardson *et al.*, 1991; Kulkarni *et al.*, 1992; Kulkarni and Fingerman, 1992; Sarojini *et al.*, 1995; Ragunathan and Arivazhagan, 1999; Vaca and Alfaro, 2000).

The larval culture of an aquatic organism is the most critical and important stage in an economic farming operation. The future success of shrimp farming will depend upon increasing supplies of healthy, high quality seeds for stocking the culture ponds. The technological developments on the large-scale culture of shrimps have been significant all over the world ever since the successful rearing of *P. japonicus* by Hudinaga (1942). The quality of larval batches produced differs immensely even under the best husbandry regimes, and the role that genetic and environmental factors play in determining larval quality is far from understood. Of late, there is a great awareness in the importance to optimise culture conditions in larviculture. The increasing interest has stimulated a series of investigations during the past two decades on the environmental requirement of culture species and salinity, being the foremost factor influencing the growth and survival of younger stages, has received considerable attention.

Chapter 1

MORPHOLOGY OF FEMALE REPRODUCTIVE SYSTEM AND OVARIAN DEVELOPMENT IN *PENAEUS MERGUIENSIS*

MORPHOLOGY OF FEMALE REPRODUCTIVE SYSTEM AND OVARIAN DEVELOPMENT IN *PENAEUS MERGUIENSIS*

Much attention has been given in recent years to understand the dynamics of reproductive process in penaeid shrimps. This is mainly to develop effective technologies for their reliable reproduction in captivity and to improve the egg and larval quality in culture practices. However, this aspect requires systematic research on fundamental aspects of the physiology and genetics. Penaeid shrimps exhibit major differences in their reproductive biology, though the reproductive system generally remains more or less the same. Owing to the quantum of energy involved and the dramatic fashion in which the ovary matures, the female reproductive system and oogenesis have been very often subjects of detailed investigation.

Morphology of the reproductive system is well documented by several crustacean workers (Chandran, 1968; Pillai and Nair, 1970; Komm and Hinsch, 1987a, b). In penaeid shrimps similar studies have been conducted by Hudinaga (1942), King (1948), Shaikhmahmud and Tembe (1958), Cummings (1961), Oka (1967), Tuma (1967), Duronslet *et al.* (1975), Anderson *et al.* (1984), Mohamed (1989), Tan-Fermin and Pudadera (1989), Dall *et al.* (1990), Hossain *et al.* (1991), Quinitio *et al.* (1993), Joseph (1996), Sandoval Quintero and Gracia (1998), Palacios *et al.* (1999) and Sakaji *et al.* (2000).

Studies on the developmental stages of gonads and the determination of practical indices are essential to understand the reproductive activity of penaeid shrimps. Among the different approaches to study the reproductive cycle of a penaeid shrimp, the external appearance or weights of the ovaries are often used for staging ovarian development. Many authors have differently reported the number of maturity stages in penaeid shrimps. Oka and Shirahata (1965) classified ovarian maturation in *P. orientalis* into 8 stages. Yano

(1988) further demonstrated 10 stages of oocyte development in *P. japonicus*. Oocyte development was classified into 4 stages in *P. monodon* (Tan-Fermin and Pudadera, 1989), *P. chinensis* (Matsuyama and Matsuura, 1983) and in *P. indicus* (Quinitio and Millamena, 1992). Thus, the number of maturity stages reported among most crustacean groups is not consistent. However, in penaeids the maturity stages generally fall under five groups – immature, early maturing, late maturing, mature and spent (Rao, 1967). Study of changes in the ratio of gonad size to body weight, known as Gonado-Somatic Index (GSI), is considered as an alternative method of assessing gonadal development (Subrahmanyam, 1963; Rahaman, 1967; Pillay and Nair, 1971). However, both the appearance of the ovaries and GSI provide only limited information on actual changes taking place in the ovaries and on the development of the oocytes. A more accurate assessment of ovarian development can be obtained by following oocyte development (oogenesis) histologically.

Development of oocytes is a critical process in the reproductive cycle of females, which involves a sequential series of cellular, physiological and biochemical events in prospective germ cells leading to the production of mature ova. The cytological studies together with morphological changes during ovarian maturation form a concrete framework for the assessment of maturity. Information about cytological changes, which occur during gametogenesis in Arthropoda, is extensive (Adiyodi and Adiyodi, 1970). Recently, research on shrimp maturation has been focused on understanding the basic mechanism involved in oogenesis or the deposition of nutrient reserves in the ova, which has a direct and measurable effect on the larval survival (Quackenbush, 1991). Oogenesis has been dealt microscopically and histologically in several species including *Penaeus japonicus* (Hudinaga, 1942; Yano, 1988), *P. setiferus* (King, 1948; Duronslet *et al.*, 1975; Chow *et al.*, 1993), *P. indicus* (Subrahmanyam, 1965; Rao, 1967; Hossain *et al.*, 1991; Quinitio and Millamena, 1992; Mohamed and Diwan, 1994), *P. merguiensis* (Tuma, 1967), *P. semisulcatus* (Bose, 1995), *P. monodon* (Motoh, 1978; Tan-Fermin and Pudadera, 1989; Dall *et al.*, 1990; Menasveta *et al.*, 1993; Quinitio *et al.*, 1993; Joseph, 1996), *P. duorarum* (Cummings, 1961),

P. aztecus (Duronslet *et al.*, 1975), *P. orientalis* (Oka and Shirahata, 1965), *P. brasiliensis* (Sandoval Quintero and Gracia, 1998), *P. kerathurus* (Medidna *et al.*, 1996), *P. paulensis* (Cavalli *et al.*, 1997), *Parapenaeopsis stylifera* (Shaikhmahmud and Tembe,1958; Shaikhmahmud, 1961; Joshi *et al.*, 1982), *Metapenaeus affinis* (Pillay and Nair, 1971), *M. dobsoni* (Vasudevappa,1992), *Metapenaeopsis dalei* (Sakaji *et al.*, 2000), *Sicyonia brevirostris* (Kennedy *et al.*, 1977), *S. ingentis* (Anderson *et al.*, 1984) and *Pandalus kessleri* (Quinitio *et al.*,1989). Electron microscopic studies on oogenesis have also been reported in *P. aztecus* (Duronslet *et al.*, 1975), *P. setiferus* (Duronslet *et al.*, 1975; Chow *et al.*, 1993), *P. indicus* (Mohamed and Diwan, 1994), *P. semisulcatus* (Bose, 1995) and in *P. monodon* (Joseph, 1996).

The germinal epithelium in crustaceans constantly produces oogonial cells mitotically during the entire reproductive life span of the female (Adiyodi and Subramoniam, 1983). Oogenesis in crustaceans can be divided into several distinct phases. Initially, there is a proliferation phase during which the germ cells divide mitotically giving rise to the oogonial cells. This is followed by a differentiative phase where the immature oocytes divide meiotically and undergo a chain of nuclear and cytoplasmic changes and an apparent increase in size. Once the oogonial cells enter meiosis, they differentiate into previtellogenic cells referred to as oocytes and protein synthesis begins within the oocytes, which then gets encompassed by follicular cells (King, 1948). The changes in the oocytes occur in a graded manner with accumulation of ribosomes and rough endoplasmic reticulum in the primary oocytes during the previtellogenic phase. This is followed by the primary vitellogenesis, during which the oocytes accumulate vitellin of ovarian origin and is characterised by an increase in egg diameter. This phase ceases when the oocytes reach certain diameter, which is characteristic of the species. Secondary vitellogenesis ensues prior to reproduction when further oocyte growth becomes synchronised. Vitellogenin, the lipovitellin precursor of the yolk protein vitellin, is taken up from the haemolymph into the oocyte by endocytosis. The final phase of oogenesis, oocyte maturation, is characterised by germinal

vesicle breakdown (GVBD) and the resumption of meiosis. This reproductive process appears to be regulated at the time of secondary vitellogenesis (Papathanassiou and King, 1984; Charniaux-Cotton, 1985; Charniaux-Cotton and Payen, 1988).

Vitellin synthesis in crustaceans, the most extensively studied aspect, has been reported to occur at both intra- and extra-ovarian sites. The proposed sites include haemocytes of the haemolymph, follicle cells, hepatopancreas and subepidermal adipose tissue (Adiyodi and Subramoniam, 1983; Eastman-Reks and Fingerman, 1985; Paulus and Laufer, 1982; 1987; Tom *et al.*, 1987; Quackenbush and Keeley, 1988; Rankin *et al.*, 1989; Quinitio *et al.*, 1989,1990; Quinitio and Millamena, 1992; Chang *et al.*, 1993a, b, 1994; Chang and Shih, 1995; Sagi *et al.*, 1995). However, some are of the opinion that ovaries are the prime sites with only a small amount being produced in extra ovarian sites (Lui *et al.*, 1974; Lui and O'Connor, 1977; Dehn *et al.*, 1983; Eastman- Reks and Fingerman, 1985; Fainzilber *et al.*, 1992). Crustaceans thus, probably employ multiple and species-specific strategies of yolk protein synthesis (Lee *et al.*, 1997).

Although vitellogenesis has been investigated in various decapods, there is no information available regarding the physical development of the ovaries in various stages of maturation in *P. merguiensis* from Indian waters. Hence, the present investigation was carried out to study the processes occurring during the reproductive cycle of *P. merguiensis* to unravel the mysteries of gonadal development and maturation. The objectives of the study include:

1) To study the morphology of female reproductive system of *P. merguiensis*.
2) To study changes in the gonadosomatic index with advancement in ovarian maturation and
3) To study the histomorphological features of oocytes at each stage of oogenesis by light microscopy.

MATERIALS AND METHODS

Specimens of *P. merguiensis* were collected on a monthly basis for 2 years (January 1998- December 2000) from trawl and gill net operations at Karwar. The collected animals were transported to the laboratory where they were segregated according to the sex. Total length (TL), carapace length (CL) body weight (BW) and gonad weight (GW) were measured to the nearest mm/ g in females. The thelycum was opened and the presence or absence of spermatophore was ascertained. The female maturity stages were classified into a five-point scale (Rao, 1967) based on gross morphological changes, gonadosomatic index and histological examinations. The steps followed in the process are listed below:

1) *Dissection and fixation :* The females in different stages of maturity were recognised on the basis of the gross morphological changes. Ovaries were carefully dissected out to study its morphology. Dissection was carried out in normal saline. The ovaries were weighed to the nearest gram and the goandosomatic index (GSI) was calculated by using the formula Gonad weight / Body weight x 100. From the monthly collections, two to five individuals (including the one, which indicated the highest GSI) were used for the histological observation of the ovary.

The process of oogenesis was studied using histological techniques with normal haematoxylin and eosin staining. Since preliminary examinations showed no difference in the state of ovarian development between anterior, middle and posterior sections of the ovary, samples were routinely dissected from the ovary in the region of the first abdominal segment of each female and fixed in Bouins fluid for 24-48 hours (Bell and Lightner, 1988) for routine histological studies.

2) *Processing and sectioning :* Tissue preparation for light microscopy was performed according to Bell and Lightner (1988). All tissues were washed overnight in running tap water to remove the excess picric acid. The tissues were then dehydrated using alcohol series (30% to 100% propanol) and cleared in chloroform. The tissues were further cold impregnated overnight with wax using a mixture of chloroform and wax shavings in a ratio of 1:1. Subsequently, the solvent was evaporated by placing the tissues in an oven at 58^0C.The tissues were then transferred through two changes of fresh molten wax. Tissue blocks were prepared using paper boats.

Serial sections of the blocks were cut at approximately 6-8µ thickness using a rotary microtome. Sections were then affixed to clean glass slides using fresh Mayer's egg albumin and flattened by placing them on a slide warmer with a drop of distilled water. Subsequently, the water was drained off and the slides were allowed to air dry. These slides were then used for histological staining.

3) *Staining :* Representative 5-6 µ paraffin cross-sections of ovarian tissues were subjected to routine staining for gross morphological observations. They were stained with Haematoxylin with 1% alcoholic eosin as the counter stain following standard procedures for general histology. Sections to be stained were deparaffinized in two changes of xylene and then dehydrated through a descending series of propanol grades. Sections were made blue using tap water or ammonia solution. Eosin stained sections were repeatedly washed in an ascending series of propanol grades to remove excess eosin and cleared in xylene. Sections were mounted with DPX and examined under a monocular microscope. Carl- Zeiss Light Microscope attached with digital camera (Axio Cam camera) was used for photomicrography.

Stages of oocyte development were designated according to the criteria adapted from Bell and Lightner (1988). The diameters of about 100 oocytes

in each stage were measured randomly with an ocular micrometer after calibration with stage micrometer (ERMA). The long axis in successive sections of an oocyte was used for the measurement of diameter.

RESULTS

A total of 600 gonads collected from female *P. merguiensis* in different developmental stages were analysed.

Morphology of Female Reproductive System

The reproductive system in females of *P. merguiensis* consisted of paired ovaries, oviducts and a single thelycum. The mature ovaries were situated dorsally extending from the base of rostrum to the last abdominal segment. They were bilaterally symmetrical and partly fused. Each half of the ovary consisted of three lobes of which the slender anterior lobe occupied the cephalic region and situated in close proximity with oesophagus and cardiac region of the stomach. The middle lobe had 6 finger-like lateral lobules, which entirely filled the area between the epigastric tooth and the posterior border of the carapace. The lateral lobules were located dorsal to the mass of hepatopancreas and ventral to the pericardial chamber. The posterior lobes of the ovary extended the entire length of abdomen, bifurcated at the end and traversed through the tissue towards the telson. The two halves of the ovary were united by two commissures, one at the base of the anterior lobe and other at the tip of the posterior lobe in the sixth abdominal segment. The thin oviducts started from the tip of the penultimate lobules of the middle lobe on either side and extended towards the external gonophore on the third pereopod (Plate 2a, b).

PLATE 2a. Lateral view of fully mature ovary showing the oviduct

PLATE 2b. Dissected out fully mature ovary with oviduct attached to 3rd pereopod

Maturation Process In *P. merguiensis*

a) Gross morphological changes in ovaries

Observation of shrimp samples collected regularly from the trawlers revealed that females of 123-125mm in total length and above (25-36mm in carapace length) exhibit indications of ovarian maturation. The commencement of maturation could be recognized externally by careful observation of ovarian changes through the arthropodial membrane between cephalothorax and abdomen. Based on the size and colour of the ovaries, five development stages viz., immature (Stage I), early maturing (Stage II), late maturing (Stage III), mature (Stage IV) and spent (Stage V) were categorised. This cycle was repeated in the reproductive phase of this animal. Size of the ovary increased simultaneously with changes in ova brought about by the maturation process. In the initial stages of maturation, the small anterior lobes and the lateral lobules of the middle lobe began to enlarge in size. At full maturity the anterior lobes grew almost up to the base of the rostral crest while the lateral lobules occupied the available space in the cephalothorax cavity. The posterior lobe also increased in thickness as maturation progressed (Plate 3).

During the immature stages the ovaries were thin, translucent and confined to the abdominal region. It consisted of developing anterior lobes, which was restricted to the posterior half of the cephalothorax. The posterior ovarian lobes situated on the dorsal aspect of the abdomen, extended up to the middle of the sixth abdominal segment. Diameter of ovarian lobe was much smaller than diameter of adjacent gut.

In the early maturing stages, ovaries increased in size with developing anterior and middle lobes; the anterior lobes developed further compared to the earlier stage and extended forward in the cephalothorax. The

PLATE 3. Females of *Penaeus merguiensis* at different stages (I - V) of ovarian maturation

I
II
III
III
IV
V

middle lobes together with their rudiment lobules also underwent further development. The posterior ovarian lobe increased in girth during this stage. The diameter of ovarian lobes was more or less similar to the diameter of adjacent gut. The general colour of the ovary was light yellow to yellowish green.

In the late maturing stages, the ovaries developed further in size with the anterior, middle and posterior lobes being fully formed. However, the anterior and middle lobes did not fill the cephalothoracic region completely. The ovary was generally light green in colour and was visible through exoskeleton. The diameter of ovarian lobe was larger than that of adjacent gut.

The fully matured ovaries were dark green in colour and clearly visible through the exoskeleton. The anterior and middle lobes were fully developed and occupied the space available in the cephalothorax. The posterior lobes developed extensions (rhomboid shape) in the first abdominal segment region. The diameter of ovarian lobe was much larger than that of adjacent gut. This stage is the last stage of maturity before actual spawning, as larger ova were encountered in this stage.

The spent ovaries were flaccid, very much convoluted and appeared whitish in colour. Variable quantities of matured ova undergoing resorption were present during this stage. It is probable that after extrusion of eggs, the gonad revert almost immediately to the immature condition. This stage was, therefore, distinguishable from immature virgin females based only on the size of the shrimp.

b) Gonadosomatic Index (GSI)

Vitellogenic activities in ovaries were estimated on the basis of ovarian weights. Stages II, III and IV were the major stages of ovarian growth as demonstrated by their two-fold increase in GSI. The oocyte diameters also increased with correspondence to the GSI value and ovarian stage. The GSI

increased during vitellogenesis reaching a maximum value at full maturity (Stage IV), but decreased after spawning to reach levels similar to immature females (Stage I). The mean GSI was found to be 0.3(± 0.2) in stage I which showed a gradual increase to 2.86 (± 2) in stage II and 5.74 (± 2.5) in stage III. The highest value of GSI was observed in stage IV (8.42 ± 2.2) followed by a steep decline in spent stage with a mean value of 0.7(± 0.3), higher than in immature females (Table 1).

c) Cytological changes

The ovarian histology of *P. merguiensis* was typical of that described previously for other penaeids. Histological preparations of the ovaries revealed the graded manner in which oocytes developed and accumulated yolk with maturation. Based on histological features five distinct stages have been identified throughout ovarian development of *P. merguiensis*. This classification was based on the presence of the most advanced oocyte stage in the ovary and the changes evident in the cytoplasm and nucleus of the oocytes. The five stages of maturity were previtellogenic, early vitellogenic, late vitellogenic, mature and spent oocytes. These oocyte phases corresponded to the maturity stages I – V classified on the basis of morphological features and colour of the ovary.

A thin ovarian wall, with two distinct layers of epithelial cells and with a layer of connective tissue between the two, encompassed the ovary. In the cross section, the outer layer of pavement epithelium was moderately basophilic with haematoxylin and eosin staining, while the inner layer composed of loose connective tissue, was moderately eosinophilic. Blood capillaries were also noticed in the peripheral regions of the ovary. The germinal zone or " zone of proliferation" was observed as a thin band along the innermost layer of the ventral ovarian wall. As the oogonial cells in the layer divided, they were pushed away from the germ cells towards the margin of the ovaries and the resultant previtellogenic cells were referred to as oocytes, which were surrounded in nodules or cyst by follicle cells.

Table 1: Characteristics of ovarian development in banana prawn Penaeus merguiensis.

Ovarian stage	Ovarian colour	Reproductive stage	GSI	Oocyte diameter (μ) of the dominant group
I	Translucent	Previtellogenic	0.3 (± 0.2)	36.11 ± 12.48
II	Light yellow to yellowish green	Early vitellogenic	2.86 (± 2)	136.66 ± 22.06
III	Light green in colour	Late vitellogenic	5.74 (± 2.5)	166.70 ± 22.72
IV	Dark green	Vitellogenic	8.42 (± 2.2)	264.95 ± 16.67
V	Whitish	Spent	0.70 (± 0.3)	24.36 ± 12.48

The smaller primary oogonial cells were found at the periphery of the germinal zone and possessed a large conspicuous nucleus, which stained lightly with haematoxylin and eosin, while cytoplasm was palely eosinophilic. The nucleoli number was high, ranging from 10-15. The secondary oogonial cells formed by the mitotic division of the primary oogonial cells (POC) were seen towards the centre of the ovary in a graded manner, the nucleus and cytoplasm of which stained lightly with haematoxylin.

Progression toward the margin was concomitant with an increase in size, the latter due in part to the nutrient provided by the follicle cells. The zone of proliferation was observed to persist in all maturity stages such as immature, early maturing, late maturing and ripe ovaries. As the oogonia passed through the developmental stages of primary oocyte and secondary oocyte, they moved from the "zone of proliferation" towards the centre of the ovarian lobe. Because of the rapid multiplication of cells, from this point the oocytes were forced to the peripheral regions of the ovarian lobe. As development proceeds, the peripheral and more mature oocytes were surrounded with "nurse" or follicle cells, which appeared to arise from the germinal epithelium. Follicle formation continued in the ripe ovary until these nutritive cells enclosed the mature ova. According to the structure and arrangement of different types of germ cells in the ovary, the microscopic scale to determine the different maturation stages were as follows :

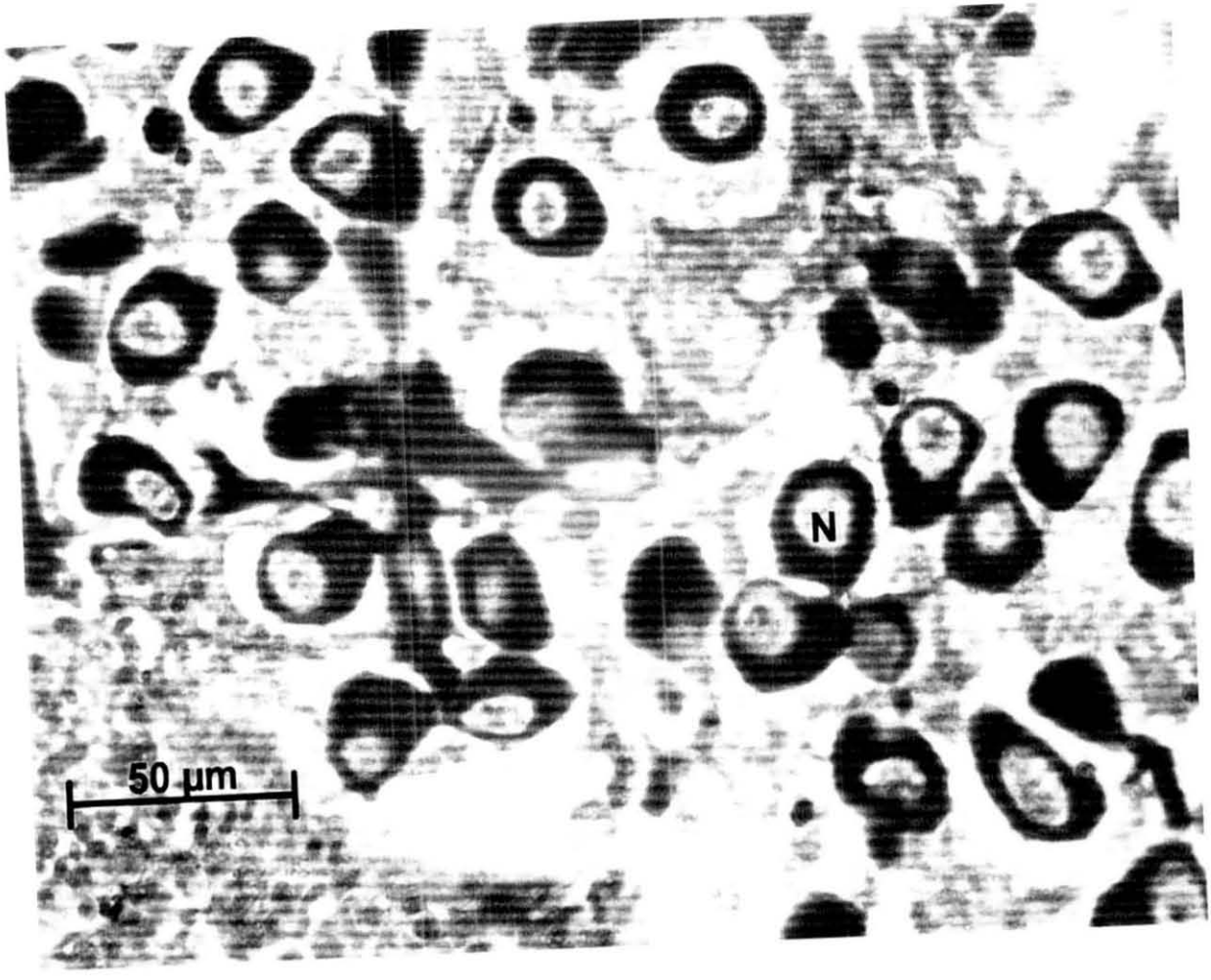
Pre-vitellogenic oocytes **(PVO):** This stage was characterised by the predominance of oogonia, differentiating oogonia and previtellogenic oocytes in the periphery of the ovarian lobe. Primary or previtellogenic oocytes (36.11 $\pm$ 12.48μ in diameter) were formed by the meiotic division of the secondary oogonial cells after which they entered meiotic prophase of the first reduction division. Two distinct size categories of previtellogenic oocytes were recognised - the smaller oocytes at the centre of the ovary whereas the larger oocytes appeared in the follicles located more peripherally. The spherical oocytes were characterised by the

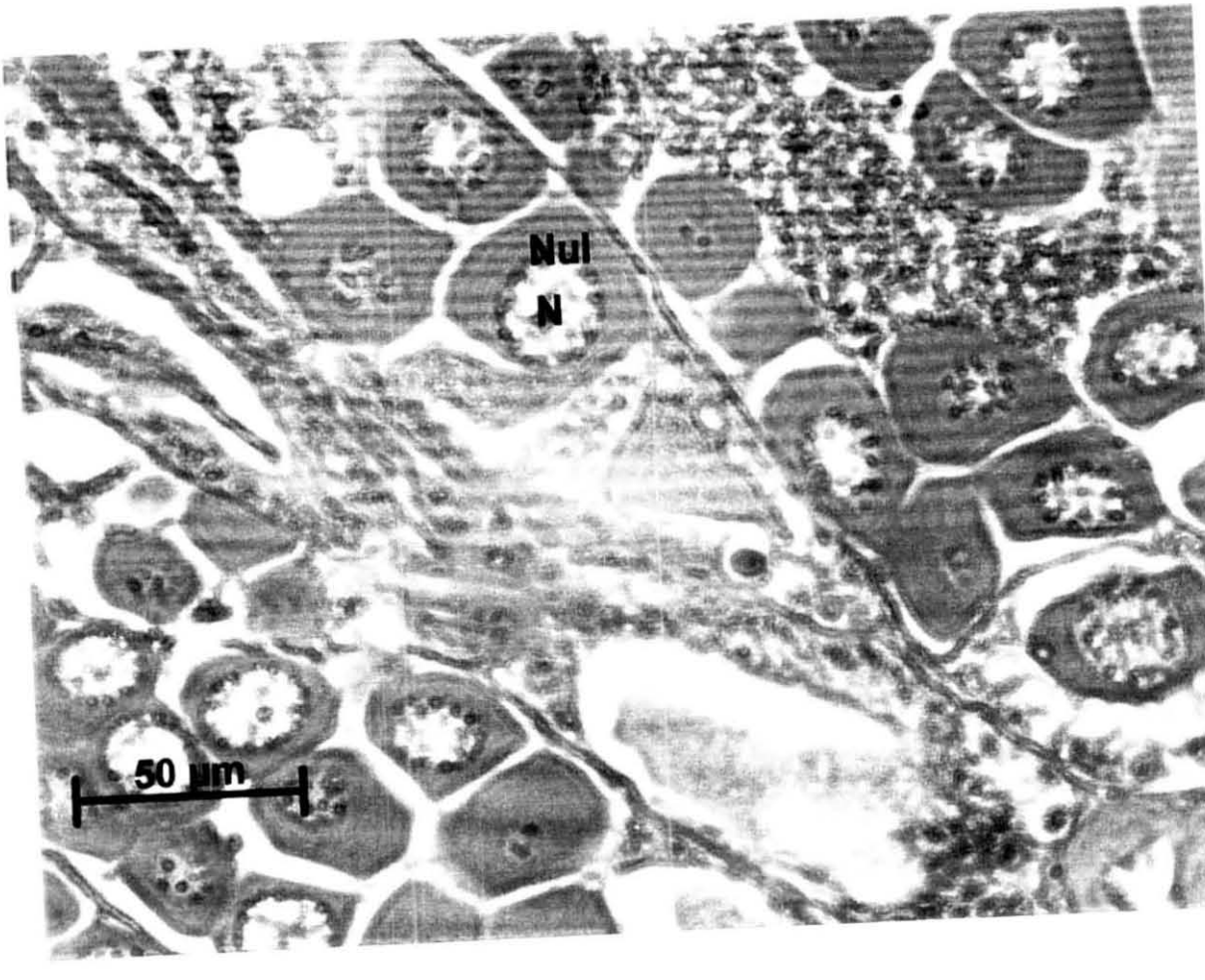
presence of agranular cytoplasm with an increased cytoplasmic volume than that of oogonial cells. The oocyte membrane was not well defined, but the centrally located lighter stained prominent nucleus (20-30μ. in diameter) had a well-defined membrane and had numerous strongly basophilic nucleoli. The nucleoli were found uniformly distributed in the germinal vesicle in small oocytes, but marginally located in larger ones. Weakly basophilic ooplasm appeared around the germinal vesicle. Oogonia were scattered in groups throughout a loose meshwork of connective tissue (Plate 4a). The pre-vitellogenic oocytes, occupied the entire lumen of the ovary in clusters surrounded by follicle cells. These follicle cells had large, oval nuclei adjacent to the follicle cell basal lamina. They were the only accessory somatic cells in the crustacean ovary and were rarely found inside the lobes.

Early vitellogenic oocytes **(EVO):** Ovaries displaying oocytes at early vitellogenesis were characterised by the presence of areas of intense mitotic activity and had a significant increase in cell size. Oogonial multiplication occurred at this stage. The oocytes (136.66 ± 22.06μ diameter) at this stage were less basophilic and slightly granular cytoplasm in contrast to the agranular and basophilic cytoplasm of the previous stage. The oocyte membrane was well defined. The volume of cytoplasm increased several fold. The granular nature and increase in volume was due to the presence of vesicular primary yolky substances in cytoplasm. Deposition of yolk in cytoplasm increased as development advanced. A large, round nucleus with a perinuclear halo of nucloelar material arranged as a circular ring around the periphery of the nucleus was observed in this phase. The ooplasm and germinal vesicle were weakly basophilic (Plate 4b). Follicle cells had begun to migrate into the ovarian lobes and appeared to be in the process of enveloping individual oocytes (folliculogenesis). A complete single layer of basophilic follicle cells (3- 5μ thick) was visible on the outer surface of the oocyte. Small groups of pre-vitellogenic oocytes were also present, but the number of vitellogenic oocytes predominated.

PLATE 4a. Stage I ovary with deeply vitellogenic oocytes having a prominent centrally placed nucleus (N – Nucleus) (x 100)

PLATE 4b. Early vitellogenic ovary showing perinucleolar material, granular cytoplasm and centrally placed nucleus (N – Nucleus; Nul – Nucleolus) (x 100)


N
50 µm


Nul
N
50 µm

***Late vitellogenic oocytes* (LVO):** During this phase, the oocytes further increased in size with ova diameter of 166.7 ± 22.72 μ and the nucleus diameter ranging from 40 to 70μ. The oocytes were round to oval in shape, with a nucleus containing several nucleoli marginally. The germinal vesicle was weakly basophilic. Characteristic feature of this phase was the very granular eosinophilic ooplasm, the volume of which had increased owing to the accumulation of yolky materials. The granular nature of the cytoplasm was mainly due to the formation of dense yolk platelets and accumulation of lipid globules. The yolk appeared as a variety of variably staining vacuoles randomly dispersed in the ooplasmic matrix and its deposition increased as development advanced. The nucleus was lightly stained with haematoxylin. The oocytes at this stage were individually surrounded by follicle cells and hence these follicle cells appeared as a narrow band of flattened cells. As the oocytes mature, the nucleus tended to lose its round appearance and the nuclear cytoplasmic ratio decreased (Plate 5). Thus the nucleus appeared much smaller. Nucleolii were still present. These yolky oocytes were preferentially located in the outer regions of the ovary. Oogonia were located in the central region of the ovary, with basophilic oocytes among them (Plate 6a).

***Vitellogenic / Mature oocytes* (MO):** Mature ovaries were characterised by the presence of fully mature oocytes that surpassed 200μ in diameter (264.95 ± 16.67 μ in diameter; nuclear diameter of 60 to 90μ). As a typical feature, the cytoplasm became highly eosinophilic, characterised by the presence of abundant yolk platelets filling the entire ooplasm and conspicuous elongated rod-like cortical bodies on its peripheral margin adjacent to the oolemma. The nucleus stained faintly and its membrane partly disappeared. The nucleoli were not apparent at this stage. The follicle cells were further flattened and were present only as a thin covering (not easily distinguishable from the oocytes which they envelope) (Plate 6b). Each cortical rod was approximately 2.4-4.5μ in diameter, appeared first near the periphery of the plasma membrane and extended towards the nucleus as vitellogenesis progressed, measuring 40μ in length and with 3.9μ and 7.4μ basal

PLATE 5. Details of late vitellogenic oocytes illustrating granular cytoplasm (N – Nucleus; Nu – Nucleolus; YG – Yolk granules; FC – Follicle cells) (x400)

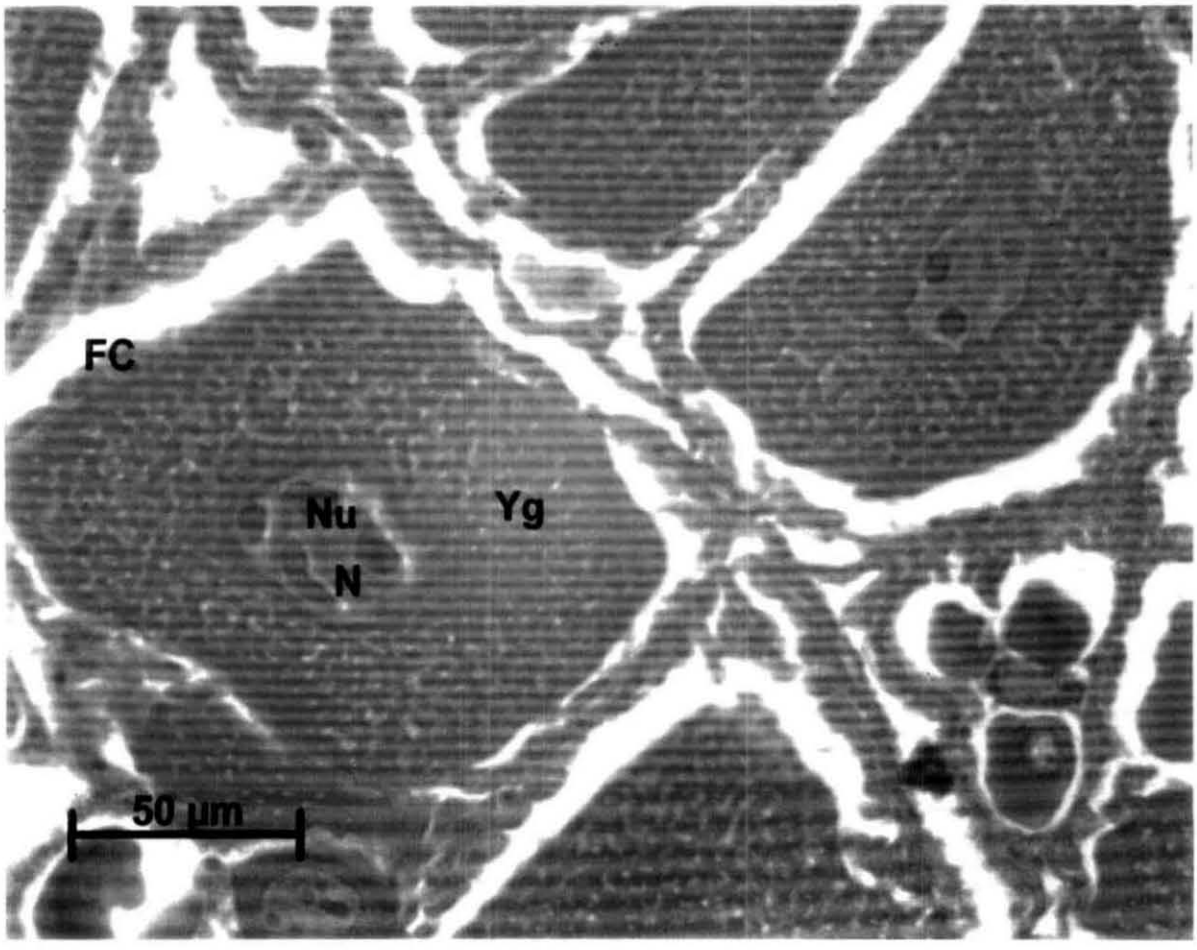
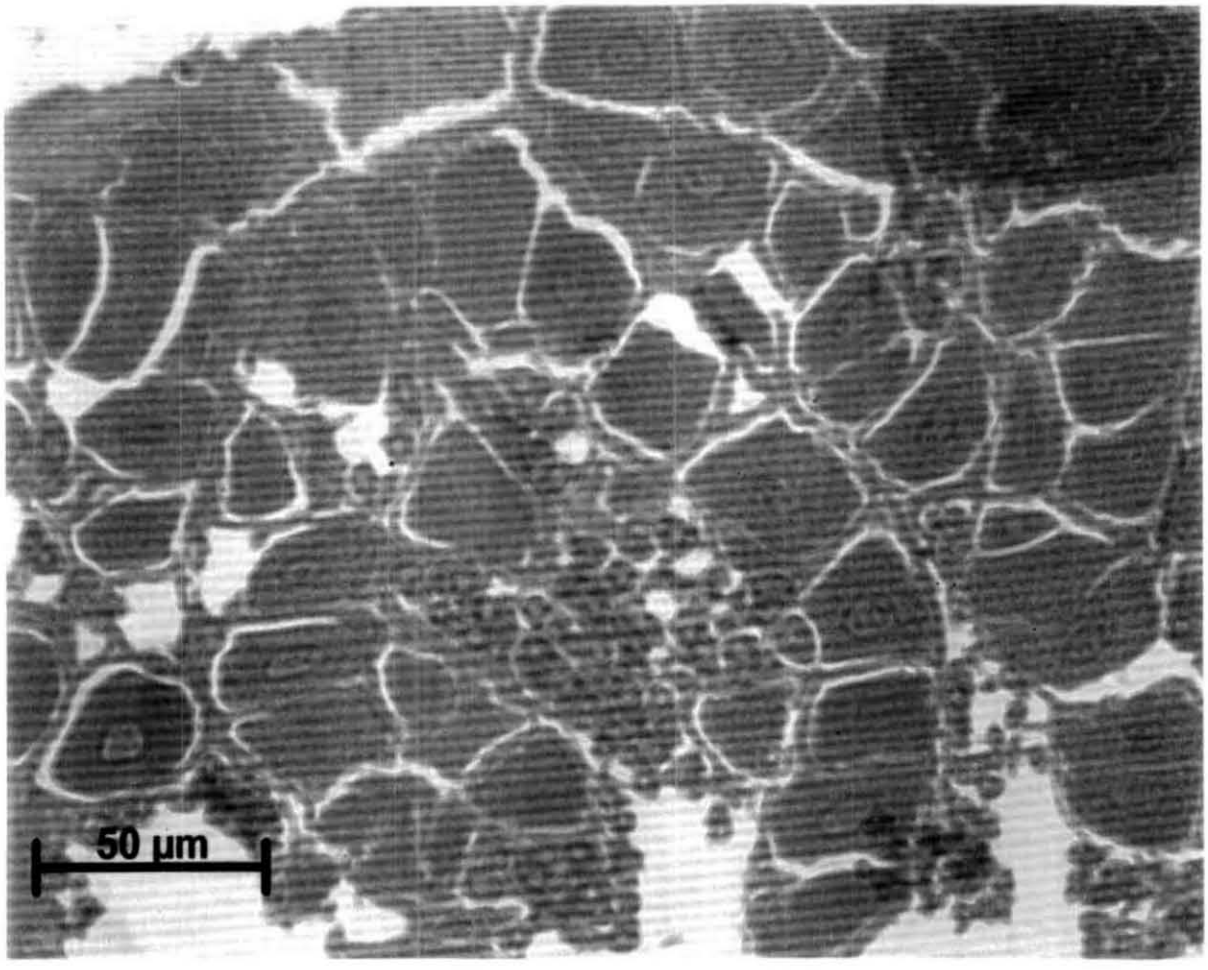
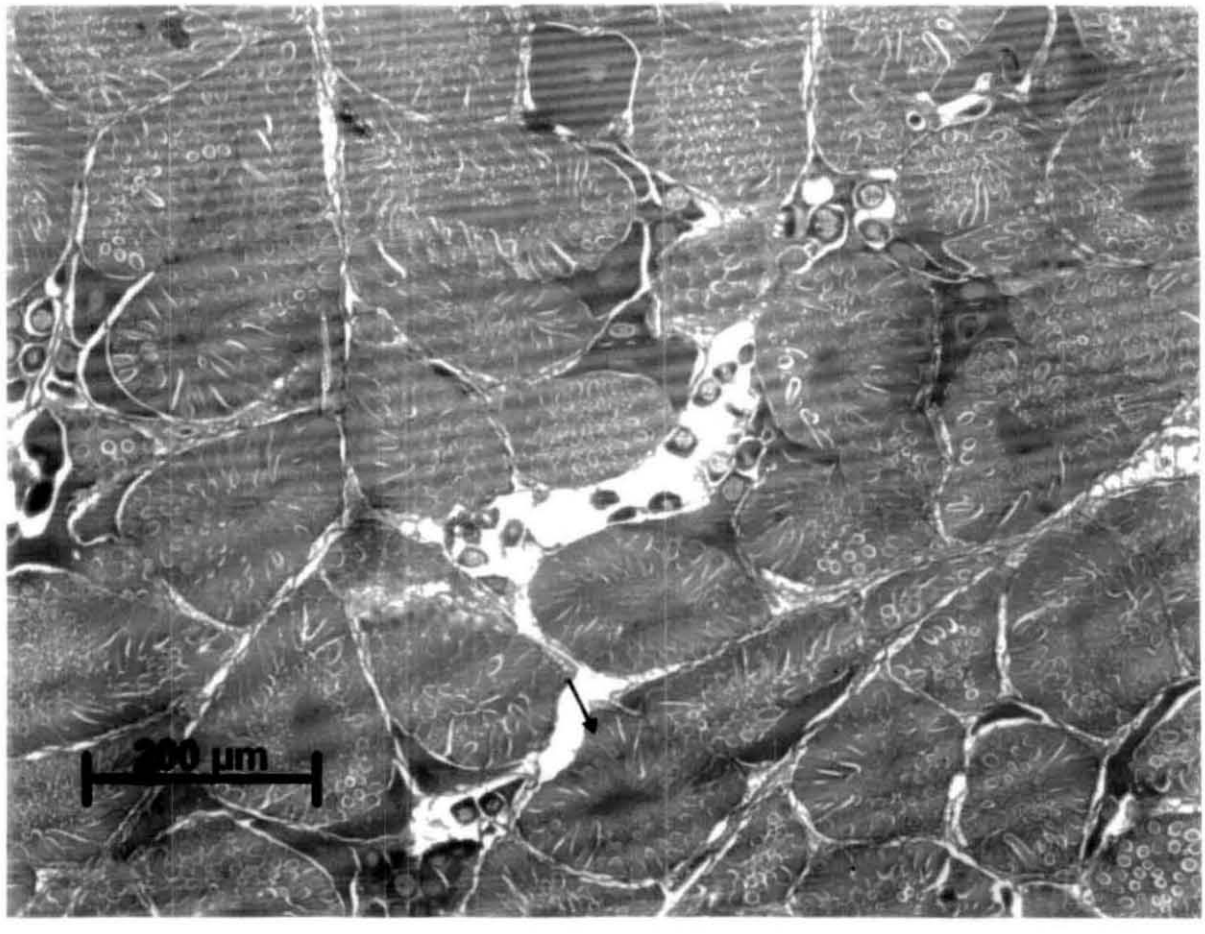

FC
Nu
N
Yg
50 µm

PLATE 6a. Stage III ovary showing late vitellogenic oocytes (x100)

PLATE 6b. Ripe ovary (Stage IV) illustrating vitellogenic oocytes (x100)


50 µm

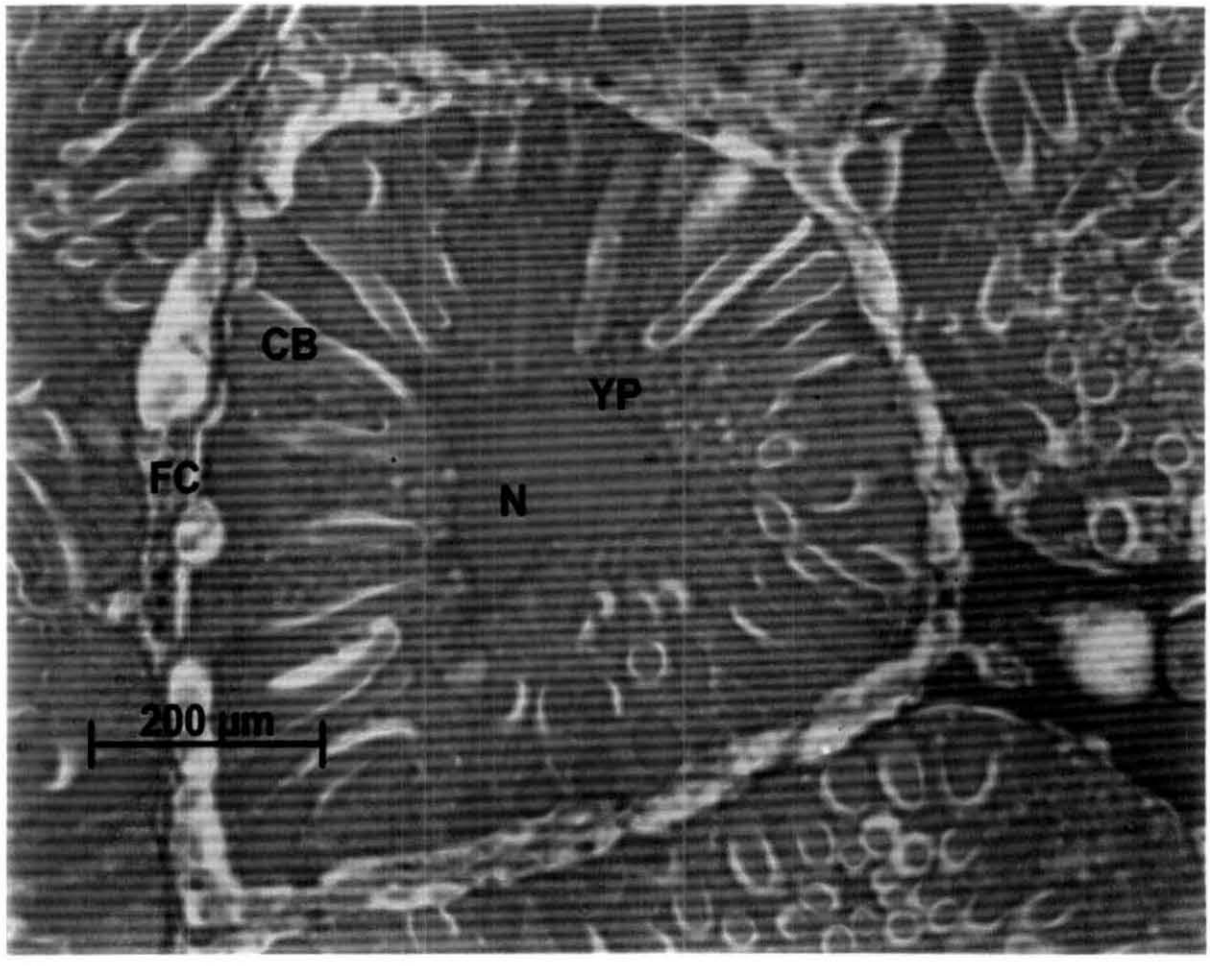

200 µm

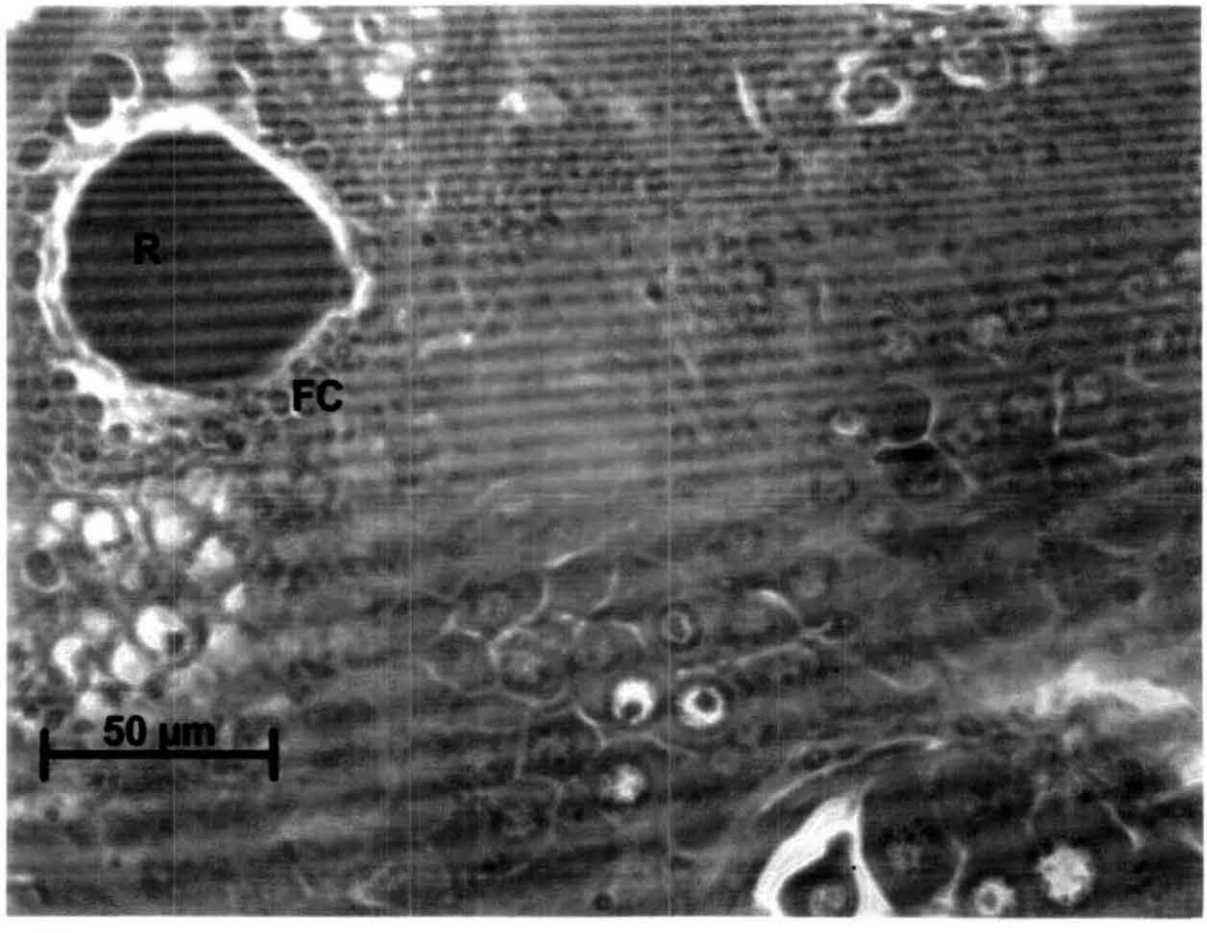
and apical diameters respectively (Plate 7a). The cortical rods were found less eosinophilic while ooplasm was eosinophilic. The yolk globules present in the ooplasm apparently indicated the period of ovulation. Oogonia and basophilic oocytes occupied the same position as in the previous stage inside the ovary.

***Spent oocytes* (SO):** This is a degenerative process by which the oocytes in various stages of development and differentiation were unrecognisable in the ovary. Oocytes of spent shrimps showed characters identical to those of previtellogenic and early vitellogenic oocytes. Microscopically, the spent ovary displayed empty follicles, sites of oocyte resorption and areas of proliferative growth of oocytes. Primary oocytes, oocytes with yolky substances and some scattered residual cortical rods were also seen. Empty follicles from which ova were released had hypertrophied follicle cells. Atypical cells which stained intensely with haematoxylin and eosin, but lacked distinct structures such as nuclei and yolk bodies were present. The structure of the atypical oocyte is consistent with the hypothesis that they are degenerating (atretic). Atypical cells were not observed in ovaries prior to stage V. Follicle cells were numerous and common at this stage and was formed by the retraction of the follicle cells from the oocytes during ovulation. The oocytes measure 24.36 ± 12.48μ in diameter. Partially spawned oocytes apparently indicated that the mature ova were undergoing breakdown and subsequently got resorbed due to its unsystematic orientation of the rod-like peripheral body. Follicle cells were found surrounding these oocytes (Plate 7b).

PLATE 7a. Cross section of fully mature oocyte showing the lightly stained nucleus and cortical bodies
(N - Nucleus; CB – Cortical bodies; YP – Yolk platelets; FC - Follicle cells) (x400)

PLATE 7b. Spent ovary (Stage V) illustrating resorbing oocyte
(R – Resorbing oocyte; FC-Follicle cells) (x400)


CB
YP
FC
N
200 µm


R
FC
50 µm

DISCUSSION

The present investigation was attempted to understand the fundamentals of various aspects of ovarian maturation in *P. merguiensis*. The morphology of female reproductive system of *P. merguiensis* was similar to that described in other penaeid species such as *P. japonicus* (Hudinaga, 1942), *P. setiferus* (King, 1948), *P. duorarum* (Cummings, 1961), *P. indicus* (Subrahmanyam, 1963; Mohamed and Diwan, 1994), *P. setiferus* and *P. aztecus* (Duronslet *et al.*, 1975), *P. monodon* (Solis, 1988; Joseph, 1996) and *Parapenaeopsis stylifera* (Shaikhmahmud and Tembe, 1958). Evidently the general structure of the female reproductive system in penaeids appears to be homologous with little inter-specific variations.

Maturity stages in penaeid shrimps generally fall into five groups, suggesting that in general, the formation and growth of female gametes or oogenesis in different species of penaeids are very similar (King, 1948; Tuma, 1967; Oka and Shirahata, 1965; Subrahmanyam, 1965; Rao, 1967; Linder and Cook, 1970; Duronslet *et al.*, 1975; AQUACOP, 1977a, b; Matsuyama and Matsuura, 1983; Primavera, 1985; Tan-Fermin and Pudadera, 1989). In this study, a relationship between the changes in gonad morphology and structure (colour, GSI and histology) during reproductive cycle in *P. merguiensis* were elucidated. The ovarian maturity stages in *P. merguiensis* were classified into five stages *viz.*, previtellogenic, early vitellogenic, late vitellogenic, vitellogenic and spent oocyte stage and this resembled those of *P. monodon* (Bell and Lightner, 1988), *P. indicus* (Subrahmanyam, 1965; Rao, 1967; Mohamed and Diwan, 1994) and that reported by Tuma (1967) in *P. merguiensis*.

Anatomical observations in the present study revealed that the course of ovarian development in banana shrimp was indicated approximately by the change in colour of the ovary from pale buff or yellow during stage II with a GSI of 2.86 ± 2 to olive- green during stage III (GSI 5.74 ± 2.5) and dark green during stage IV (GSI 8.42 ± 2.2). In stage I, fresh ovaries were translucent and therefore difficult to observe while viewing through the abdominal terga. As vitellogenesis proceeds, oocytes matured synchronously with the accumulation of yolk and also developed characteristic green colour as a result of deposition of carotenoid pigments. This green colour of the shrimp ovary, which is an indicator of vitellogenesis and responsible for the dark outline of the ovary, may be due to the pigment ovoviredin, as in the lobster *Homarus americanus* (Talbot, 1981). The classification (stage I – V) of ovarian development on the basis of external characterisation correlated closely to the oocyte diameter, ovarian growth (GSI) and ovarian histology. This classification could have a practical application for broodstock management in shrimp hatchery (Sagi and Ra'Anan, 1985).

Histological characterisation of the maturation stages in *P. merguiensis* in the present study revealed the presence of two protective layers encompassing the ovary. Similar observation was made by King (1948) in *P. setiferus* and by Tuma (1967) in *P. merguiensis*. The germinal zone (ventromedian in position), from which the oogonial cells moved away giving rise to oocytes, was observed to be present in ovaries in all maturity stages in *P. merguiensis* indicating that the ovary was active throughout the reproductive period of the female. Identical observations have also been made in other penaeids such as *P. setiferus* (King, 1948) and *P. japonicus* (Yano, 1988). As oocytes developed further, they migrated out towards the margin of the ovarian lobes in preparation for ovulation.

Primary and secondary vitellogenesis in *P. merguiensis* gave rise to secondary oogonial cells and vitellogenic oocytes respectively as reported in other penaeids. Vitellin is the major reserve material in the oocyte, which gets

accumulated in yolk globules in penaeid shrimps (Tom *et al.*, 1987). Increase in size of developing oocytes appeared to be the main feature allowing separation of maturing stages. Large areas of stage I ovaries were covered by intracellular space. Stage II ovaries contained many basophilic previtellogenic cells. The previtellogenic oocytes served as a temporal indicator of oocyte maturation during oogenesis and "set the stage" for the ensuing events associated with yolk production (Komm and Hinsch, 1987a,b). The basophilic nature of cytoplasm of the ova of pre and early vitellogenic oocytes gradually shifted to the acidophilic nature in the late vitellogenic and vitellogenic oocytes in *P. merguiensis*, which was similar to that noticed in *P. setiferus* (King, 1948), *P. monodon* (Tan-Fermin and Pudadera, 1989), *P. indicus* (Mohamed and Diwan, 1994) and *M. dobsoni* (Vasudevappa, 1992). During the process of oogenesis, the number of nucleoli got reduced and finally in the vitellogenic stage they were not apparent. The yolk platelets or globules were densely accumulated in the cytoplasm of *P. merguiensis* in mature oocytes. This almost agreed with the observation made by Mohamed and Diwan (1994) in *P. indicus*, Browdy *et al.* (1986, 1990) in *P. semisulcatus* and Joseph (1996) in *P. monodon*.

The appearance of oil globules in the cytoplasm represented the onset of late vitellogenesis in *P. merguiensis*. Mohamed and Diwan (1994) reported similar observation in *P. indicus*. The first appearance of oil globules in the cytoplasm has been interpreted differently in various decapods. It was suggested that the appearance of oil globules represented the initial stage of primary vitellogenesis in *P. japonicus* (Yano, 1988) and in the red frog crab *Ranina ranina* (Minagawa *et al.*, 1993), but in *Panulirus japonicus* it was viewed as the initial stage of vitellogenesis (Minagawa and Sano, 1997). In contrast, oil globules were not mentioned at all in a description of vitellogenesis of the crayfish *Procambarus clarkii* by Kulkarni *et al.* (1991). Abdu *et al.* (2000) reported the first appearance of oil globules in the oocyte cytoplasm of *Cherax quadricarinatus* to mark the last stage of primary vitellogenesis.

Cortical rods (previously referred to as jelly-like substance, cortical specializations, rod-shaped bodies, cortical crypts, marginal bodies and peripheral bodies, etc.) existed in all mature specimens observed in Penaeidae and Sicyonidae (Hudinaga, 1942; King, 1948; Oka and Shirahata, 1965; Tuma, 1967; Duronslet *et al.*, 1975; Anderson *et al.*, 1984; Tan *et al.*, 1985; Yano, 1988; Tan- Fermin and Pudadera, 1989; Medidna *et al.*, 1996). They do not occur in the ovaries of *Parapenaeopsis stylifera* (Shaikhmahmud and Tembe, 1958), *Metapenaeus ensis* (Yano, 1985), *M. dobsoni* (Vasudevappa, 1992) and *Metapenaeopsis dalei* (Sakaji *et al.*, 2000). In the present study, the rod-shaped bodies in the peripheral cytoplasm of mature ova of *P. merguiensis* were apparent only during stages IV and V. The cortical rods characteristics of genus *Penaeus* were indicators of imminent spawning (Anderson *et al.*, 1984). The present study indicated that cortical crypts were found initially in the peripheral part of the oocytes and later extended towards the nucleus. Similar results were reported in other penaeids shrimps (Hudinaga, 1942; King, 1948; Cummings, 1961; Duronslet *et al.*, 1975; Yano, 1988; Joseph,1996).

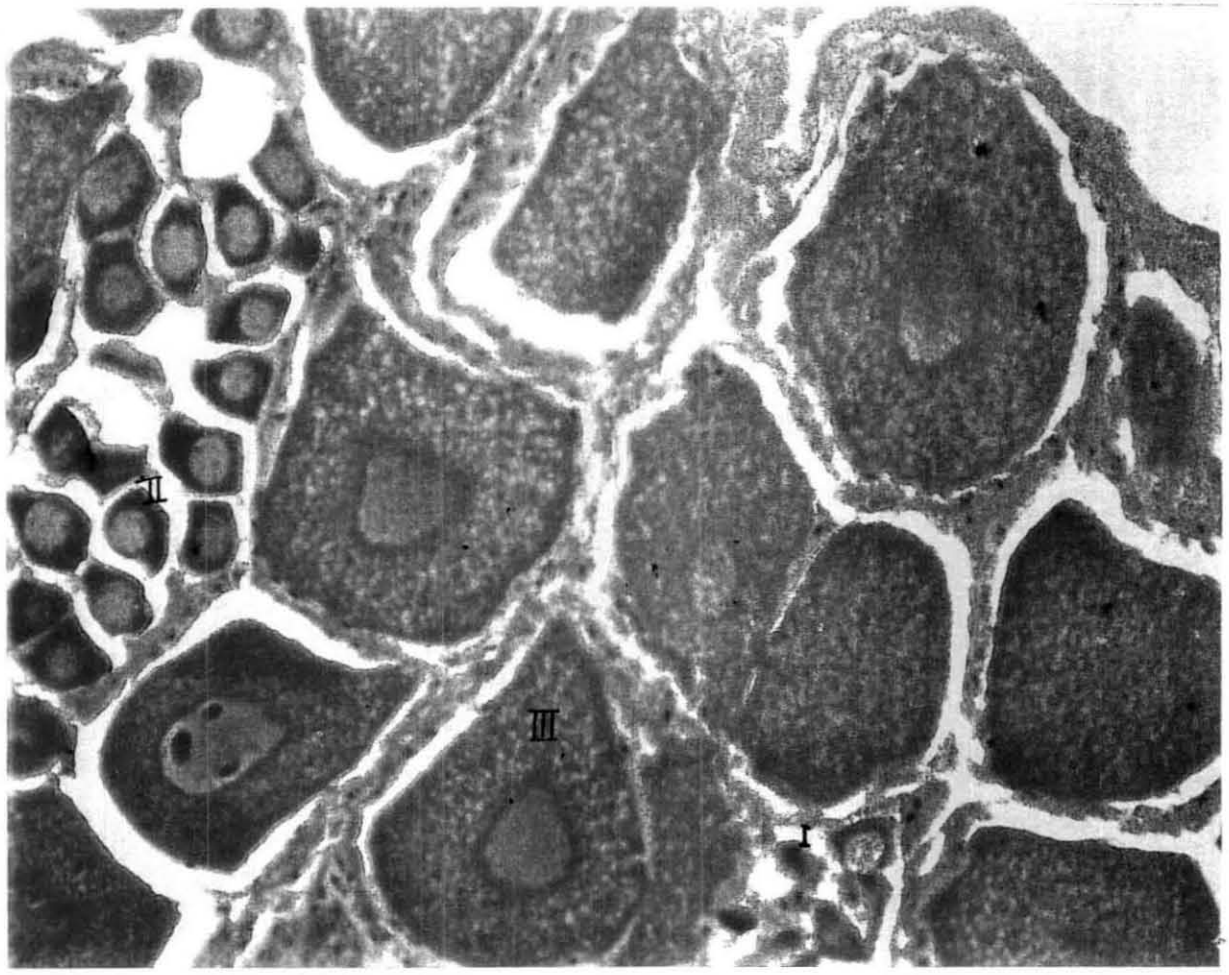
In penaeid shrimps the precursor of cortical granules began to develop by the end of secondary vitellogenesis. The function of these rod-shaped bodies is not known though Hudinaga (1942) reported the simultaneous disappearance of rod shaped bodies and appearance of egg-jelly surrounding the egg in *P. japonicus* immediately after spawning. Cortical bodies were reported to be invaginations of the oolemma and responsible for the jelly layer, which surrounds the egg on exposure to seawater during early development in shrimps (Clark and Lynn, 1977; Anderson *et al.*, 1984; Clark *et al.*, 1990). As a result of the extrusion of the cortical rods upon exposure of spawned eggs to seawater, the egg reduced its volume in comparison to ripe oocytes inside the ovary. Their function is still undetermined, though they are believed to play an important role in the early development (Lynn *et al.*, 1991). Most of the accumulated vitellin was reported to be synthesized by the penaeid ovary itself (Fainzilber *et al.*, 1992) as well as the cortical crypts proteins (Bradfield *et al.*, 1989, Rankin *et al.*, 1989). The culmination

of ovarian development was marked by release of the fully mature oocytes (eggs) into the oviduct at ovulation.

It was observed that different stages (Stages I - III) of oocytes development were present in the same section of the ovary in addition to the synchronously growing oocytes (Plate 8). The presence of very few oocytes with cortical rods after spawning showed that wild *P. merguiensis* spawners release almost all of their eggs at a time. The darkly stained irregularly shaped primary oocytes found in the spent ovaries may be oocytes squeezed when the advanced ones were expelled through the oviducts. The presence of mostly small, immature oocytes in the newly spent ovaries indicated that it might take more duration for a wild *P. merguiensis* to remature and spawn in a breeding season. Generally, the simultaneous existence of different developmental stages of oocytes in the ovary indicated that the species might be a multiple spawner (Sakaji *et al.*, 2000). Multiple spawning in natural conditions in a single spawning season has been reported in *P. merguiensis* in the Gulf of Carpentaria (Crocos and Kerr, 1983) and also in *P. latisulcatus* in Western Australia (Penn, 1980).

Follicle cells were large, round, basophilic and more obvious in the previtellogenic and early vitellogenic oocyte (stage II), which was consistent with the studies by Chang and Shih (1995) and O'Donovan *et al.* (1984) in *Macrobrachium rosenbergii* and Tuma (1967) in *P. merguiensis.* In *P. merguiensis,* folliculogenesis or the investment of follicular cells around the oocytes was initiated in previtellogenic oocytes and completed by early vitellogenic phase. Follicle cells were not easy to identify in late vitellogenic and mature oocytes. Yano and Chinzei (1987) suggested that vitellogenin was synthesized in the follicle cells of *P. japonicus.* The size of follicle cells might reflect their biosynthetic ability. The flattening of the follicle cells with the growth in volume of the oocyte observed in the present study was characteristic of the process. Hudinaga (1942), King (1948) and Oka and Shirahata (1965) in describing ovarian development in various penaeids, made use of the appearance of follicle cells as a

PLATE 8. Cross section of female ovary illustrating different (stages I – III) stages of oocyte development (x100)


II
III
I

means of separating ovarian stages of maturity. Ovarian follicles in *P. merguiensis*, the function of which is not known, behaved as described by Hudinaga (1942), *i.e.* the follicle cells diminished in size as ova size increased suggesting that ova may gradually absorb nutrients from follicle cells. The size of follicle cells was considered not to be a distinct indicator of ovarian stages of development in *P. merguiensis*. According to Charniaux-Cotton (1975), follicle cells facilitate vitellogenic activity by aiding in the uptake of yolk protein from external sources. Probably in *P. merguiensis* too, the follicle cells function in a similar manner. Yano and Chinzei (1987) suggested that vitellogenin is synthesized in the follicle cells of *P. japonicus*. The size of follicle cells might reflect their biosynthetic ability. Added evidence for the phenomenon was the observation of pinocytotic vesicles along the oolemma in late vitellogenic phase in *P. indicus* (Mohamed and Diwan, 1994) and the display of PAS positivity by follicle cells with the progress in maturation. The positivity was maximum in spent follicle cells. This indicated that apart from protein synthesis, follicle cells might also be involved in synthesis and mobilization of carbohydrate moiety of the yolk. Therefore it is probable that the follicle cells may facilitate an exogenous supply of yolky material as suggested by Varadarajan and Subramoniam (1980) in the case of anomuran crab *Clibanarius clibanarius*.

Follicular atresia is the degenerative process by which oocytes in various stages of development and differentiation are lost from the ovary (Guraya, 1973). Atretic cells were commonly found in the spent ovaries as reported in other penaeid species such as *P. indicus* (Mohamed and Diwan, 1994), *P. monodon* (Tan-Fermin and Pudadera, 1989), *M. dobsoni* (Vasudevappa, 1992) and *P. semisulcatus* (Bose, 1995).

Though the present investigation revealed that the process of oogenesis is a complex process, it also indicated that ovarian maturation in *P. merguiensis* followed the typical pattern as seen in other penaeids especially (i) the presence of rod-like cortical bodies as indicators of imminent spawning, (ii) the initiation of follicular cells in folliculogenesis and their diminishing nature as ova

size increases and (iii) appearance of oil globules in the cytoplasm with oocytes development .The results of this investigation are additional information to understand the reproductive biology of *P. merguiensis*, which are necessary for pond broodstock management technology in the shrimp hatchery.

Chapter 2

CHARACTERISATION OF VITELLOGENIN AND VITELLIN OF *PENAEUS MERGUIENSIS*

CHARACTERISATION OF VITELLOGENIN AND VITELLIN OF *PENAEUS MERGUIENSIS*

Early developmental events in arthropods are accompanied by changes in protein pattern in the haemolymph and ovaries, which are more marked among sexes towards maturity (Horn and Kerr, 1969; Croisille *et al.*, 1974; Elliott and Gillot, 1979; Meusy 1980). Knowledge of such specific events is essential for the studies of hormonal regulation in decapods. Vitellogenesis, the process of yolk deposition within the developing oocytes, is a crucial event in oogenesis of crustaceans (Byard and Aiken, 1984; Derelle *et al.*, 1986; Quackenbush, 1986; Fingerman, 1987; Tom *et al.*, 1987; Meusy and Payen, 1988; Rankin *et al.*, 1989; Browdy *et al.*, 1990; Shafir *et al.*, 1992; Chang *et al.*, 1994, 1996; Sagi *et al.*, 1995; Lee *et al.*, 1997; Qiu *et al.*, 1997; Jasmani *et al.*, 2000; Kawazoe *et al.*, 2000). It has been intensively studied in lower vertebrates (Lam *et al.*, 1978; Tata, 1978) and in various groups of crustaceans, but due to the diverse assemblage in the group, penaeids have received little attention. But of late, there has been an increasing interest in crustacean vitellogenesis, due to their growing commercial importance in aquaculture and the interest in the development of new models for basic research (Laufer and Downer, 1988).

Vitellogenesis in crustaceans is characterised by the appearance of vitellogenin (Vg), the possible precursor of vitellin, in haemolymph of vitellogenic females as a female specific protein (FSP) (Wallace *et al.*, 1967; Horn and Kerr, 1969; Fielder *et al.*, 1971; Fyffe and O'Connor, 1974; Derelle *et al.*, 1986; Suzuki, 1987; Nelson *et al.*, 1988; Chang *et al.*, 1994; Chen and Chen, 1994; Chang and Jeng, 1995a, b; Lubzens *et al.*, 1997; Lee *et al.*, 1997; Longyant *et al.*, 2000; Okumaura and Aida, 2000). Vitellogenin is generally detected as a prominent fraction of low electrophoretic mobility in the serum of vitellogenic (in Amphipoda,

Isopoda and Decapoda) and prepubertal (in Amphipoda and Decapoda) females, containing lipids, carbohydrates and phosphorus (lipo-glyco-phosphoprotein). It is mostly absent or present in very small amounts in males. Many authors have studied the electrophoretic behaviour of FSP in the haemolymph during egg formation and reported its expression to increase in the haemolymph with advancement in vitellogenesis. Reports also show that vitellogenin is at the highest concentration prior to maximum accumulation of yolk in the oocytes (Wolin *et al.*, 1973; Meusy, 1980; Dehn *et al.*, 1983; Derelle *et al.*, 1986; Marzari *et al.*, 1986; Tom *et al.*, 1987; Nelson *et al.*, 1988; Quackenbush and Keeley, 1988; Quackenbush, 1989b; Quinitio *et al.*, 1989; Shafir *et al.*, 1992; Laxmilatha, 1991; Chang and Shih, 1995; Lee *et al.*, 1997). Thus, the female specific protein (FSP) level in the haemolymph is a good indicator of the vitellogenic state of the animal.

The molecular weight of purified vitellogenin in several crustaceans was found to range between $2x10^5$ and $4x10^5$Da. The molecular weights are of the same magnitude in decapods, isopods, amphibians, fishes and birds. Though vitellogenin is usually seen as a single prominent fraction, multiple fractions have been detected in certain cases. Separation of vitellogenin by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) revealed 8 to 11 components, the molecular weights of which ranged between $0.31x\ 10^5$ and $2.35\ x\ 10^5$ daltons. In *P. monodon* vitellogenin had a molecular weight of 263kDa with two polypeptide subunits (170kDa and 82kDa) (Chang *et al.*, 1994). In contrast to this, Chen and Chen (1994) reported four subunits for vitellogenin (74, 83, 104 and 168kDa) while Longyant *et al.* (1999) reported 5 subunits (74, 83, 104 168 and 200kDa) for *P. monodon*. Chang and Jeng (1995a, b) reported two polypeptide subunits (85 and 191kDa) for vitellogenin in *P. chinensis*. Three polypeptide subunits (200, 120 and 80kDa) were reported in *P. semisulcatus* (Lubzens *et al.*, 1997). Thus, crustacean vitellogenin is apparently a composite protein fraction, the production of which is likely to be multi-gene involved and multi-hormone controlled process (Adiyodi and Subramoniam, 1983).

Vitellin (Vn), the major yolk protein that accumulates within the ovary during the process of vitellogenesis in crustaceans is the nutritive material necessary for the successful development of an embryo independent of its mother (Meusy and Payen, 1988). Penaeid shrimps release their eggs directly into the sea for fertilisation. Unlike many decapods, the nauplii of penaeid shrimp hatch without any parental care or protection (Anderson *et al.*, 1949; Cook and Murphy, 1969). During the first 36-48 hours after hatching, penaeid nauplii subsist entirely on yolk (vitellin) retained from the egg. Therefore, the quality and quantity of egg yolk is crucial for the successful early life of penaeid larvae. Vitellin is also found in other animals whose eggs have a high concentration of yolk such as insects (Hagedon and Kunkel, 1979) and amphibians (Wallace and Bergink, 1974). Because of the dense concentration of vitellin in such eggs and the relative ease with which it can be isolated and purified, it is an excellent protein to study the regulation and mechanism of oocyte development. The source, composition and production of yolk (vitellin) have been studied primarily in decapods (Adiyodi, 1985; Adiyodi and Subramoniam, 1983; Charniaux-Cotton, 1985; Quackenbush, 1991). Vitellin has more or less the same mobility as vitellogenin and also shares common immunogenic sites with vitellogenin (Croisille and Junera 1980; Meusy *et al.*, 1983; Charniaux- Cotton, 1985; Picaud *et al.*, 1989). However, the biochemical characteristics of vitellogenin and vitellin and the relationship between them including their origin, are not well understood in crustaceans.

Ovarian vitellin has been purified and characterised in several penaeid shrimps namely *P. japonicus* (Vazquez-Boucard *et al.*, 1986; Yano and Chinzei 1987), *P. vannamaei* (Quackenbush, 1989a,b; Tom *et al.*, 1992), *P. semisulcatus* (Browdy *et al.*, 1990; Tom *et al.*, 1992; Khayat *et al.*, 1994; Lubzens *et al.*, 1997), *P. chinensis* (Chang *et al.*, 1996), *P. monodon* (Quinitio *et al.*, 1990; Chen and Chen 1993; Chang *et al.*, 1993a; Longyant *et al.*, 1999), *Parapenaeus longirostris* (Tom *et al.*, 1987) and *Metapenaeus ensis* (Qiu *et al.*, 1997). Early studies on crustacean vitellin characterised it as a high molecular

weight (350kDa) protein that contained sugar, lipid and carotenoid moieties (lipo-glyco-carotenoprotein), (Wallace *et al.*, 1967; Lui and O'Connor, 1976; Adiyodi, 1985; Charniaux-Cotton, 1985). With some species, the molecular nature of vitellin is still not well documented with respect to either size or number of subunits. It appears that the molecular weight of native vitellin in most penaeids is about 300kDa. The molecular weight of native vitellin was found to be 510kDa for *P. japonicus* (Vazquez-Boucard *et al.*, 1986), 289kDa for *P. vannamei* (Rankin *et al*, 1989; Tom *et al.*, 1992), 283kDa for *P. semisulcatus* (Browdy *et al.*, 1990) and 350kDa for *Metapenaeus ensis* (Qiu *et al.*, 1997). Molecular weight of native vitellin in *P. monodon* was reported as 540kDa (Quinitio *et al.*, 1990; Chen and Chen, 1993) and also as 492kDa (Chang *et al.*, 1993a). Two forms of vitellins with molecular weights 380 and 500kDa were reported in *P. chinensis* (Chang *et al.*, 1996).

The number of polypeptide subunits in vitellin reported for penaeids is generally between 2 to 5. Browdy *et al.* (1990) reported that vitellin of *P. semisulcatus* had four subunits (50, 63, 80 and 90kDa) while only two polypeptide subunits (86 and 90kDa) were described by Tom *et al.* (1992) in the same species. In contrast, three polypeptides of molecular weights 80, 91 and 158 and 80, 120 and 200kDa were reported as vitellin subunits of *P. semisulcatus* by Khayat *et al.* (1994) and Lubzens *et al.* (1997) respectively. Vazquez-Boucard *et al.* (1986) reported five polypeptide subunits in *P. japonicus* while Yano and Chinzei (1987) reported only two subunits in the same species. Vitellin of *P. monodon* was detected to have four major polypeptide subunits *viz.*, 74, 83, 104 and 168kDa, and a minor one (90kDa) (Quinitio *et al.*, 1990; Chen and Chen, 1993) while Chang *et al.* (1993a) reported eight polypeptides (35, 45, 49, 58, 64, 68, 82, 91kDa). Eight subunits (105, 85, 78, 58 and 40kDa for Vn1 and 155, 85, 78kDa for Vn2) were elucidated for *P. chinensis* (Chang *et al.*, 1996). Tom *et al.* (1987) described two subunits for *Parapenaeus longirostris* (45, 66kDa). Quackenbush (1989a, b) reported three polypeptides (76, 97 and 158kDa) in the ovary of *P. vannamei.* In contrast to this, while Tom *et al.* (1992) reported only two

polypeptides (61, 69kDa) for *P. vannamei*. Similarly in *Metapenaeus ensis* two major subunits (76, 102kDa) were reported by Qiu *et al.* (1997).

The origin of the yolk protein varies among the decapods and the precise site of synthesis is not yet confirmed. An approach to vitellogenesis as a flow system, in which vitellogenin is produced by an extra ovarian tissue, released into the haemolymph and subsequently taken up by the developing oocytes was described in insects (Bakker-Grunwald and Applebaum, 1977). A similar conclusion has been drawn in crustaceans (Byard and Aiken, 1984). These authors suggested that FSP represents an externally synthesised protein and was found at the highest level in the haemolymph during the period when oocytes accumulates the maximum amount of yolk.

From the foregoing statements, it is evident that no studies have been conducted on vitellogenin and vitellin of *P. merguiensis* in particular. Hence, the present study was aimed to describe the changes observed in the serum and ovarian protein profile (vitellogenin and vitellin respectively) of *P. merguiensis* during maturation. Partial purification and characterisation of vitellin from the ovaries and vitellogenin from haemolymph of *P. merguiensis* were attempted to elucidate their characteristics and relationship between them.

MATERIAL AND METHODS

Selection of animals

Live female *Penaeus merguiensis* at different stages of maturity were collected from the gill nets from Karwar and Amdalli area. They were transported to the laboratory in transportation bags with oxygen and maintained in plastic pools filled with filtered seawater until used for experiments. Classification of ovarian

development was done on a five point scale based on their relative size, outline and colour of ovaries as seen through the transparent dorsal exoskeleton (Rao, 1967).

Sample preparation

The haemolymph collected from females in the late vitellogenic stage (Stage III) were used for isolation of vitellogenin while dark green coloured ovaries collected from mature (Stage IV) females were used for the isolation and partial purification of vitellin. Haemolymph was collected from the shrimps through the pericardial cavity using needles rinsed with 0.2M Ethylenediamine tetra acetic acid disodium salt (EDTA). Haemolymph from males and immature female was also collected for the purpose of comparative studies.

a) ***Haemolymph samples :*** The collected haemolymph was left undisturbed at 4^0C overnight to ensure complete clotting. The clot was loosened with a needle and clear serum was obtained by a 10-15 minute centrifugation at 10,000 g. Serum was frozen at -20^0C till use.

b) ***Ovarian homogenates :*** After haemolymph collection, the animals were sacrificed and ovaries were dissected out, weighed and freezed till use. Fragments of ovarian tissue at various stages of vitellogenesis were homogenised in equal volume of double distilled water in ice-cold condition. The homogenates were centrifuged in cold at 10,000 g for 15 minutes and the fat cap was removed before the supernatant was used for electrophoresis or for subsequent purification.

Isolation of vitellogenin fraction: Approximately 0.5ml of serum was diluted to 5ml and subjected to ammonium sulphate precipitation technique (Wallace *et al.*, 1967). The bulk of protein precipitated at 50% and 60% saturation of ammonium

sulphate under ice cold condition was found to contain vitellogenin. The precipitate obtained was centrifuged at 10,000 g for 15 minutes and the pellet thus obtained was dissolved in 200µl of distilled water and its purity was detected electrophoretically. Vitellogenin was more prominent at 60% saturation and hence, subsequent analysis of serum samples was carried out at 60% saturation of ammonium sulphate.

Isolation of Lipovitellin fraction: Approximately 0.5ml of the supernatant obtained from ovarian homogenate was diluted to 5ml using double distilled water and subjected to ammonium sulphate precipitation. Lipovitellin was precipitated at 50% saturation of ammonium sulphate under ice-cold condition and was centrifuged at 10,000g for 15 minutes. The pellet thus obtained was dissolved in 200µl of distilled water and used for further experiments. Purity of the resulting preparation was checked electrophoretically.

Electrophoresis

Haemolymph and ovarian homogenates together with their purified fractions were subjected to Native Polyacrylamide Gel electrophoresis (PAGE) as described by Davis (1964) using 1.8M Tris- HCl buffer, pH 8.3 (Table 2). Separating gel of different concentration *viz.*, 6, 6.5, 7 and 7.5% were tried and a gel concentration of 6.5% was found to be ideal giving a better resolution (Table 3). Electrophoresis was conducted at constant current of 65mA at 10^{o}C. The molecular weights of the samples were determined using standard native gel markers of known molecular weights [Urease, 272 (trimer) and 545 (hexamer)] run along with the samples.

Samples were also subjected to Sodium Dodecyl Sulphate Polyacrylamide Gel electrophoresis (SDS PAGE) according to Laemmli

Table 2. Reagents for Electrophoresis

PAGE			SDS PAGE		
Stock Acrylamide Solution			**Stock Acrylamide Solution**		
Acrylamide	29.100g		Acrylamide	29.100gm	
Bisacrylamide	0.900g		Bisacrylamide	0.900gm	
Gel Buffers			**Gel Buffers**		
Separating gel buffer			***Separating gel buffer***		
1.8M Tris	21.800gm		1.5M Tris	9.086gm	pH 8.8
Temed	250.000	pH 8.9			
Stacking gel buffer			***Stacking gel buffer***		
0.5M Tris	3.028gm	pH 6.8	0.5M Tris	3.028gm	pH 6.8
Electrode buffer			**Electrode buffer**		
Glycine	36.000gm	pH 8.3	Glycine	28.270gm	pH 8.3

(1970) with some modifications. Separating gels of 11, 11.5 and 12% were tried initially. Further analysis of samples were conducted in a separating gel of 12% using 1.5M Tris HCl (pH8.8) along with a stacking gel of 3% as it gave better electrophoretic separations without any trailing of bands (Table 3). The molecular weight of the polypeptide subunits were determined using SDS PAGE gel markers of known molecular weights namely Phosphorylase b (97.4kDa), Bovine Serum Albumin (66kDa), Ovalbumin (43kDa), Carbonic Anhydrase (29kDa), Soyabean Trypsin Inhibitor (20kDa) and Lysozyme (14kDa). Electrophoresis was conducted at constant voltage of 140V.

Sample Preparation for Loading

The concentration of protein samples to be loaded on the gel was standardised to obtain an ideal resolution. Stock of sample buffer without SDS was used for native PAGE while sample buffer with SDS was used for SDS-PAGE (Table 4).

Serum samples : Fifteen micro-litres of crude serum sample were found enough for native PAG electrophoretic determination of the protein patterns. This sample was diluted with 15µl of distilled water and mixed with 30µl of sample buffer for loading. In the case of vitellogenin fraction isolated from haemolymph using ammonium sulphate, 40µl of the extract was mixed with 30 micro-litres of sample buffer for loading.

In SDS PAG electrophoretic analyses of serum samples, 10µl of serum sample diluted to 30µl, was mixed with 30µl of sample buffer and boiled for five minutes. The resulting solution was centrifuged at 10,000g for 15 minutes and the supernatant was used for loading. Fifty micro-litres of vitellogenin fraction isolated from haemolymph by ammonium sulphate precipitation technique was

Table 3. Composition of Native and SDS PAGE Gel

Native PAGE (6.5%)		SDS PAGE (12%)	
Separating gel		**Separating gel**	
Acryalmide and bisacrylamide	10.80ml	Acryalmide and bisacrylamide	12.00ml
Separating gel buffer	6.25ml	Separating gel buffer	6.00ml
Double distilled water	8.00ml	Double distilled water	11.57ml
Ammonium per sulphate	25.00ml (400ul of fresh 1% APS diluted to 25ml)	Ammonium per sulphate	100.00ul (from 10% stock)
		10% SDS	300.00ul
		Temed	30.00ul
Stacking gel		**Stacking gel**	
Acryalmide and bisacrylamide	1.50ml	Acryalmide and bisacrylamide	2.00ml
Stacking gel buffer	30.75ml	Stacking gel buffer	2.50ml
Double distilled water	9.40ml	Double distilled water	5.40ml
Ammonium per sulphate	100.00ul	Ammonium per sulphate	40.00ul
Temed	25.00ul	10% SDS	100.00ul
		Temed	10.00ul

Table 4. Sample buffers for electrophoresis

PAGE (without SDS)		SDS PAGE (with SDS)	
Glycerol	2.00ml	Glycerol	20%
Bromophenol Blue	1.00ml	Beta mercaptoethanol	10%
Stacking gel buffer	7.00ml	Bromophenol Blue	6% of 0.1% stock
		Stacking gel buffer	18%

mixed with equal volume of sample buffer and boiled for five minutes prior to centrifugation. The supernatant was stored till use for electrophoretic analyses.

Ovarian samples *:* Forty micro-litres of ovarian sample were found to be ideal for native PAG electrophoretic analyses of molecular weights of both crude ovarian extracts and partially purified vitellin samples. The samples were mixed with thirty micro-litres of sample buffer prior to loading.

Seventy five micro-litres of both ovarian homogenate and ammonium sulphate precipitated vitellin extracts were mixed individually with equal volume of sample buffer containing SDS for determining the polypeptde subunits of vitellin. The mixture was boiled for five minutes to ensure complete interaction between proteins and SDS. The resulting mixture was centrifuged at 10,000g for 10minutes and the supernatant was used for loading.

Marker **:** Ten micro-litres of standard molecular weight markers for both PAGE and SDS PAGE (GENEI, Bangalore) were mixed with 60μl of sample buffer for loading. This mixture was boiled for a minute prior to loading in SDS PAGE.

Staining the gels : The gels, from both native as well as SDS electrophoresis, were carefully removed and subjected to an overnight fixation once the electrophoretic run was over. This was followed by staining gels for two hours in Coomassive Brilliant Blue R-250 to study the general protein profile. The excess stain was washed off and the gels were finally immersed in destainer (Table 5).

Table 5. Details of staining techniques adopted in electrophoresis

Tests	Fixation time	Staining time	Destaining solution and duration	Colour of band
Protein				
Coomassive Brilliant Blue	10% TCA for 30 minutes	30 minutes in dark	Methanol, water and acetic acid (5: 5: 1) for 30 minutes	Blue
Polysaccharide				
Periodic Acid Schiff's Reagent	12.5% TCA for 30 minutes	One hour in 1% peiodic acid in 3% acetic acid; 1 hour wash in double distilled water; 3 hour in Schiff's reagent in dark under refrigeration	1% aqueous metabisulphite followed by repeated washing in 7% acetic acid	Magenta
Lipoprotein				
Sudan Black B		2 hours	Ethanol for 30 minutes	Brownish black
Calcium				
Alizarin Red S		20 minutes	Repeated washing in double distilled water; rinse in acid ethanol (-103 M HCl in 95% ethanol) until background clear	Deep reddish orange

Characterisation of vitellogenin and vitellin

Native gels prepared to detect vitellogenin and vitellin were stained for carbohydrate, lipid and calcium moieties with Periodic acid Schiffs reagent, Sudan Black B and Alizarin Red 'S' respectively in addition to Coomassive Brilliant Blue R-250 for the general protein profile as shown in Table 5. Relative mobilities (R_f) of proteins were estimated and the molecular weights were determined in comparison with markers.

RESULTS

The changes in the protein profile of ovarian and haemolymph samples during maturation of *P. merguiensis* were resolved by native PAGE and SDS PAGE.

Identification and characterisation of Vitellogenin

Native PAGE profiles of haemolymph samples from females in different stages of maturity revealed the presence of a specific protein, which increased in intensity with advancement in maturation. This protein, considered as vitellogenin (female specific protein, FSP), was intensively expressed in females in the late vitellogenic stage (Stage III) and was present in low concentration in immature females and males. The expression of vitellogenin in serum samples was less prominent in native PAGE as it was masked by haemocyanin. Subjecting the serum samples to 60% ammonium sulphate fractionation reduced the expression of haemocyanin considerably (Plate 9). Subsequent electrophoretic analyses revealed a single form of vitellogenin of molecular weight 350kDa, which was stainable with SBB, PAS and Alizarin Red S indicating the presence of lipid, carbohydrate and calcium moieties in it (Plate 11).

SDS PAGE analysis of immature and mature female serum after ammonium sulphate fractionation showed changes in the polypeptide profile in the course of vitellogenic process. Six polypeptide subunits of molecular weights 104, 102, 99, 68, 64 and 24kDa were detected in purified vitellogenin in SDS-PAGE of which 99kDa was feebly expressed in immature serum (Plate 12; Figure 2).

Identification and characterisation of vitellin

Plate 10 shows the result of preliminary native PAGE analysis of ovarian extracts. The pale green colour of shrimp yolk was retained, which was used as a marker to follow the yolk during purification. Vitellin, the main constituent of ripe ovaries was identified by comparing the electrophoretic profile of homogenates prepared from ovaries at various stages of oogenesis. The gradual appearance of vitellin during oogenesis was clearly demonstrated by the increase in the intensity and width of the vitellin band with advancement in maturity. The protein profile from PAGE of ovarian homogenates showed the presence of a heavily stained band in stage II- IV ovaries which was weakly expressed in stage I ovary. A narrow band, which stained lightly with Coomassive Brilliant Blue, was also visible in mature females. These bands had a molecular weight of 300 and 550kDa respectively and were believed to represent vitellin (Vn1 and Vn2). The deeply stained band (Vn1) was stainable with Sudan black B, PAS and Alizarin red S (Plate 11), while the narrow band (Vn2) was stainable only with PAS. This indicates that Vn1 was lipo-glyco-calcium complex while Vn2 was glycoprotein in nature.

Plate 12 shows the result of SDS PAGE analysis of non- vitellogenic and vitellogenic ovarian extracts. 12% SDS-PAGE analysis showed that high molecular weight polypeptides were prominent in the ovaries in the late vitellogenesis, which was not discernible at the onset of vitellogenesis. Five bands of molecular weights 104, 102, 68, 55 and 4 4kDa were prominent in these

PLATE 9. Native PAGE illustrating the appearance of vitellogenin in haemolymph of females at different stages (Stage I- V) of maturity

Lane 1. Male
Lane 2. Immature female
Lane 3. Early vitellogenic female
Lane 4. Late vitellogenic female
Lane 5. Mature female
Lane 6. Spent female

→ Vitellogenin

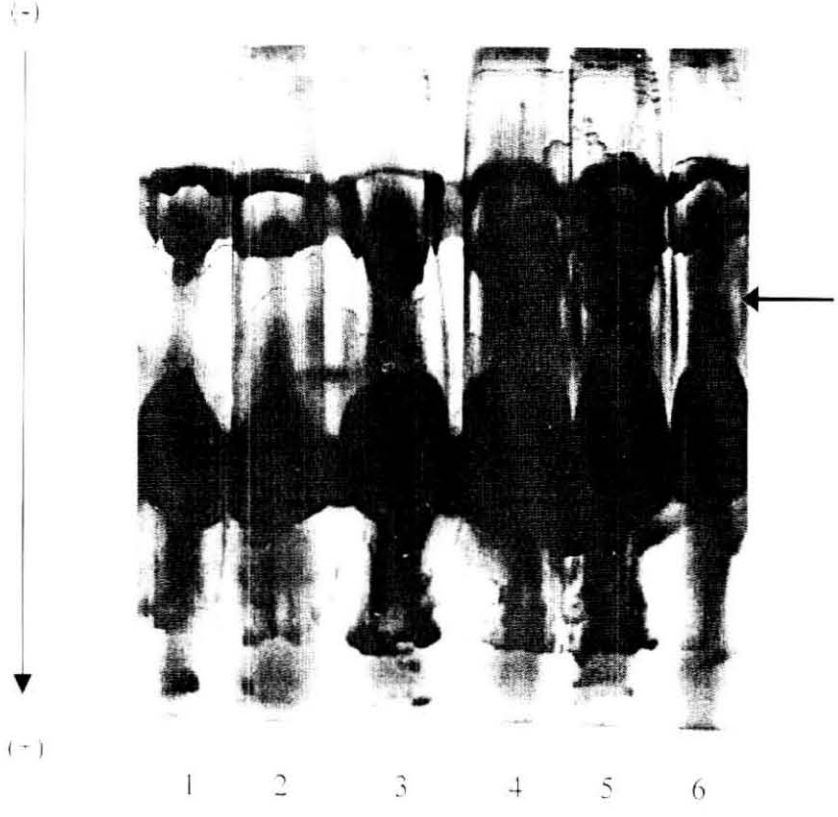

(-)
(+)
1
2
3
4
5
6

PLATE 10. Native PAGE illustrating the appearance of vitellin in ovaries of females at different stages (Stage I- V) of maturity

Lane 1. Stage I ovary
Lane 2. Stage II ovary
Lane 3. Stage III ovary
Lane 4. Stage IV ovary
Lane 5. Stage V ovary

→ Vitellin

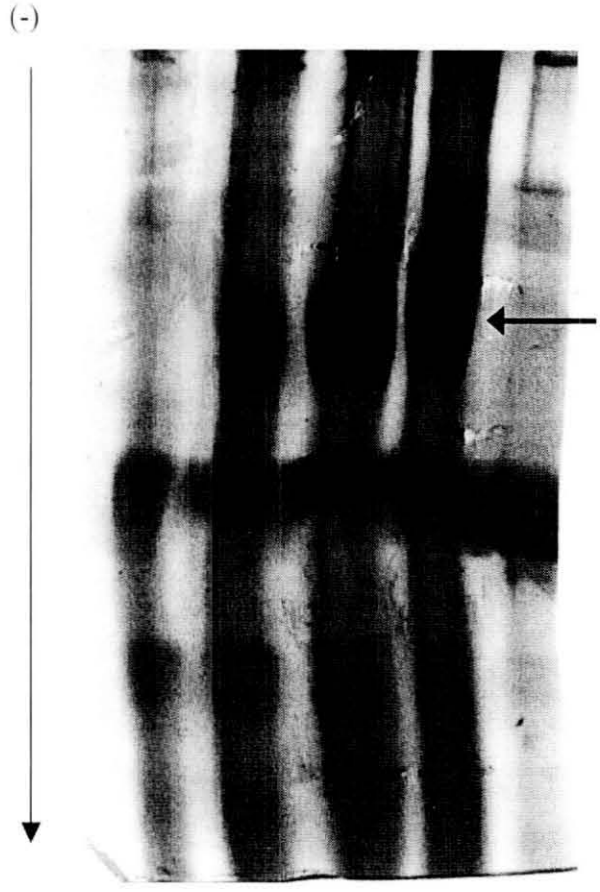
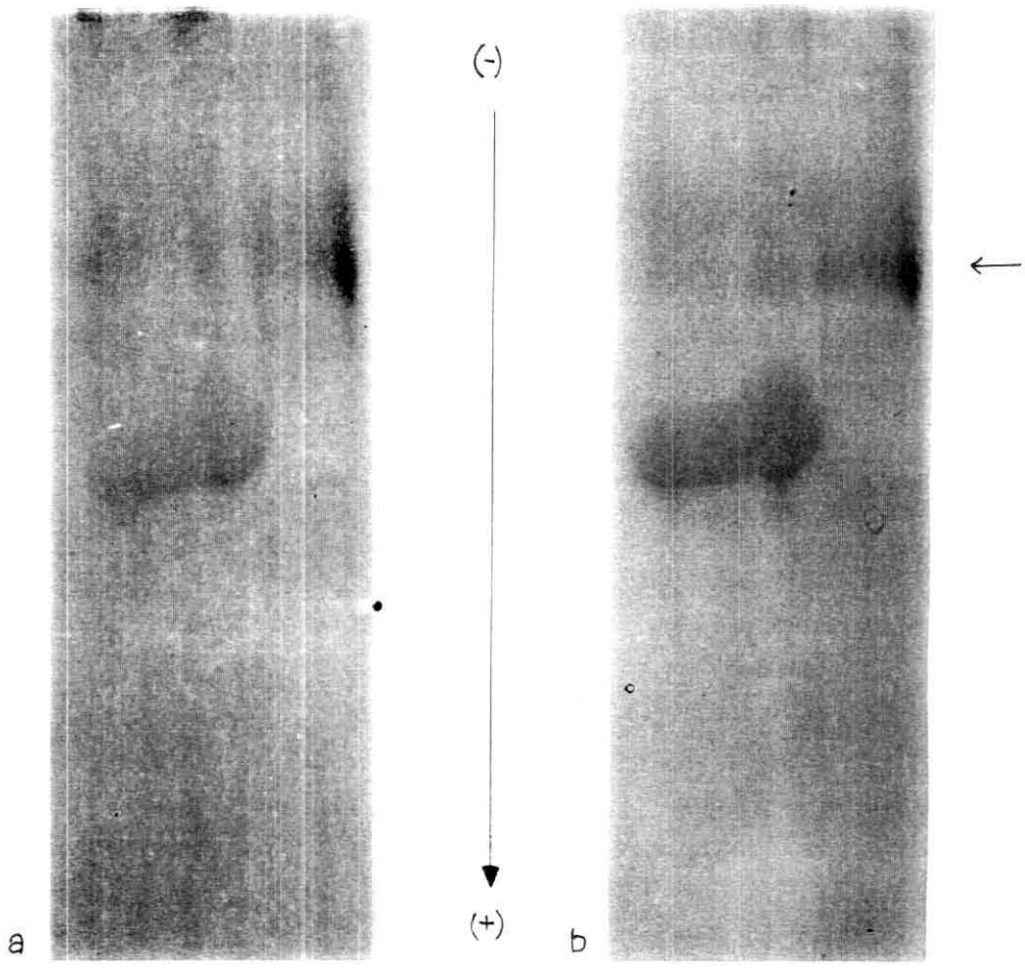

(-)
(+)
1
2
3
4
5

PLATE 11. Native PAGE of vitellogenin and vitellin stained with Sudan Black B and Alizarin Red S

a: Vitellogenin fraction stained with Sudan Black B
b. Vitellogenin fraction stained with Alizarin Red S
c. Vitellin fraction stained with Sudan Black B
d. Vitellin fraction stained with Alizarin Red S


(-)
(+)
a
b

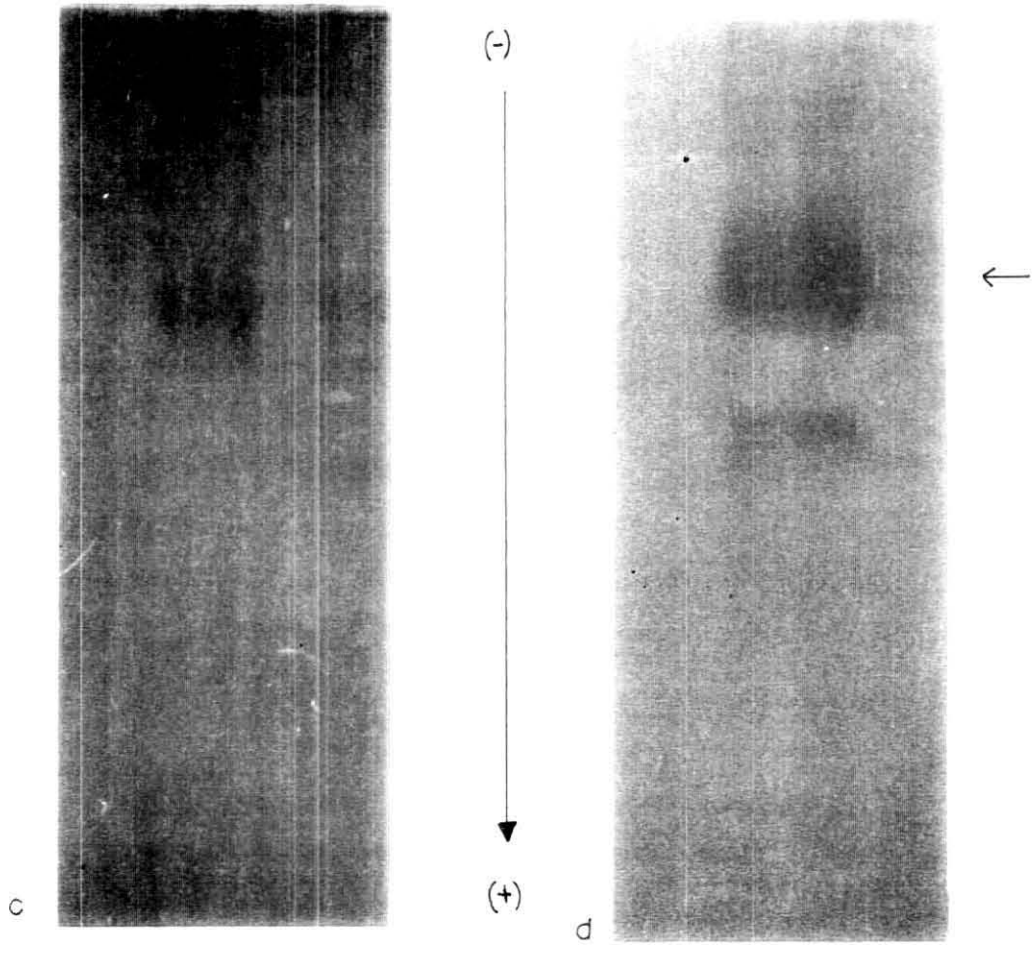

(-)
(+)
c
d

PLATE 12. SDS PAGE analysis of vitellogenin and vitellin from immature and mature females

Lane 1. Haemolymph from immature females
Lane 2&3. Haemolymph from mature females
Lane 4. Marker
Lane 5. Mature ovary
Lane 6. Immature ovary

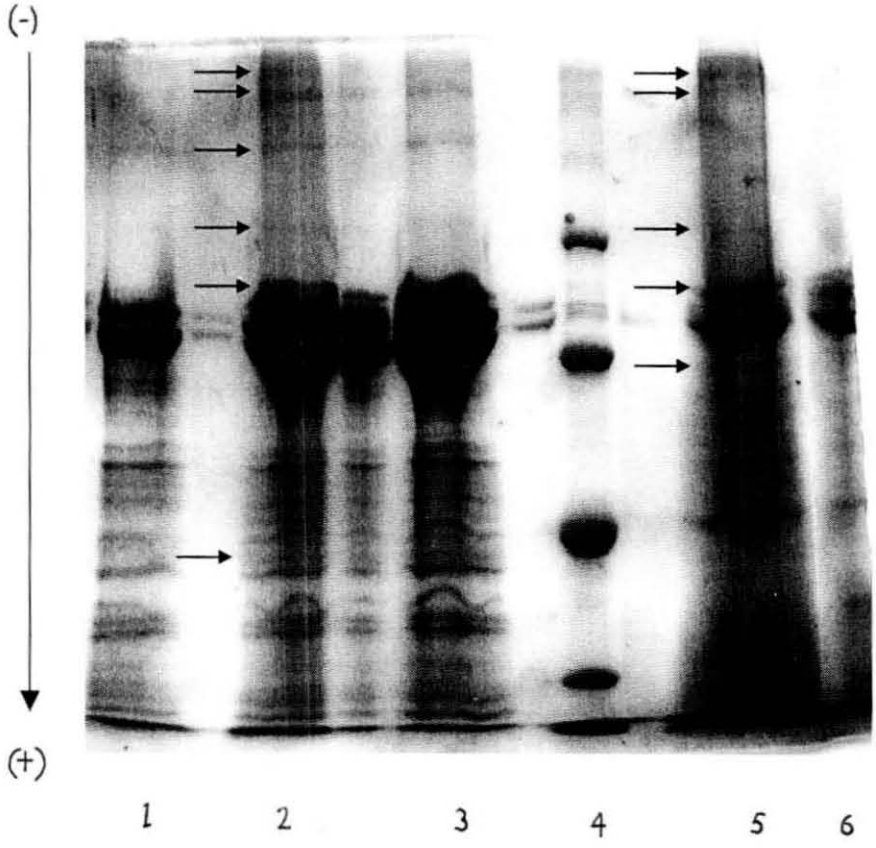
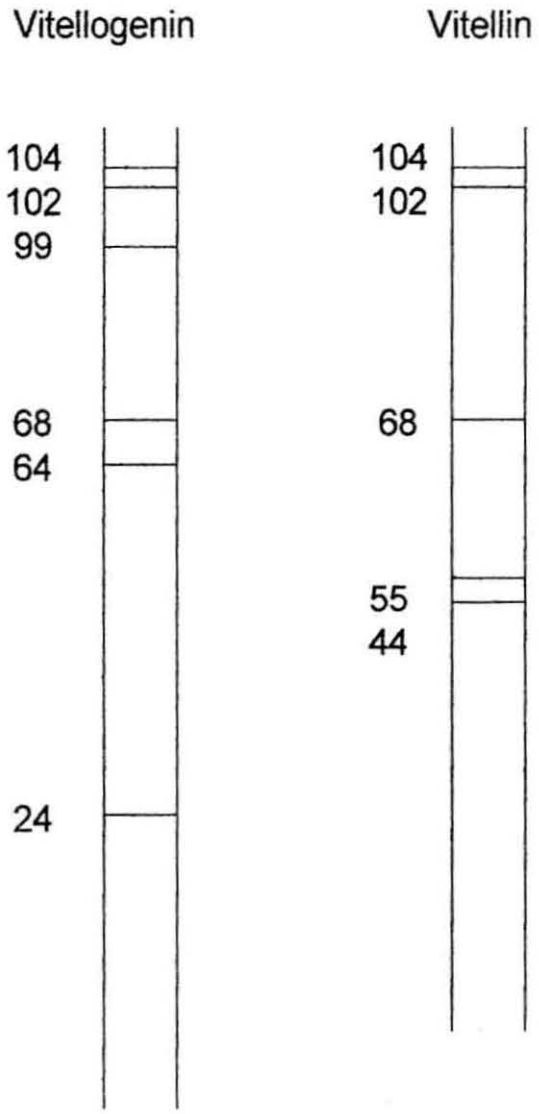

(-)
(+)
1
2
3
4
5
6

FIGURE 2. Comparative electropherograms of vitellogenin and vitellin patterns at vitellogenic stage in *Penaeus merguiensis*.



females of which only 55kDa was feebly expressed in stage I ovary (Figure 2). Some or all of these polypeptides bands represent the polypeptides that accumulate to high levels during yolk formation and thus likely to represent vitellin subunits.

DISCUSSION

Of late considerable interest has been given to studies on vitellogenesis in crustaceans. Vitellogenin, the precursor of yolk protein, is synthesised in the liver in vertebrates, released into the blood and selectively accumulated into the developing oocytes (Wallace, 1978). In invertebrates, yolk proteins are stored in oocytes with progressing maturity and thereafter the ovary develops in the same manner as in vertebrates. In general vitellogenin and vitellin are reported to be lipo-glycoproteins with calcium affinity and hence staining with Sudan black B, PAS and Alizarin Red S was resorted to identify these proteins in the present study.

Vitellogenin was identified as a prominent fraction of low electrophoretic mobility in the haemolymph of vitellogenic females of *P. merguiensis* and was present in low concentration in the haemolymph samples of males and previtellogenic females. The electrophoretic detection of vitellogenin in haemolymph was difficult due to the presence of haemocyanin having more or less same molecular weight. However, vitellogenin became more distinct after ammonium sulphate precipitation. It was observed as a prominent protein band (molecular weight 350kDa) in native PAGE and was characterised as lipo-glyco-calcium-protein based on their staining properties with a molecular weight of 350kDa. Different forms of vitellogenin were suggested in crustaceans such as the amphipod (*Orchestia gammarella*), the isopod (*Pocellio dilatatus*) and shrimp (*P. chinensis*), which had a molecular weight of 397, 315 and 500kDa respectively (Meusy and Payen, 1988; Chang and Jeng, 1995a, b). Two forms of vitellogenin

were observed in *Homarus americanus* (Nelson *et al.*, 1988). Suzuki (1987) indicated that the haemolymph of isopod (*Armadillidium vulgare*) had four forms of vitellogenin. Only one form of purified vitellogenin was detected in *P. merguiensis* in native PAGE with six polypeptide subunits. Purified vitellogenin isolated from *P. monodon* had a molecular weight of at least 263kDa with 2 subunits (82kDa and 170kDa). In the freshwater prawns *Macrobrachium rosenbergii*, vitellogenin was found to have a molecular weight of 92kDa (Derelle *et al.*, 1986; Lee *et al.*, 1997) with two polypeptide subunits (191 and 85kDa).

Concentration of vitellogenin in the haemolymph of *P. merguiensis* was found to vary during different stages of maturation. It increased dramatically during initial stages of oocyte development and was always highest well prior to the maximum accumulation of yolk in the oocytes (Stage III) and the levels decreased markedly after spawning. The increased concentration in stage III is indicated by the intense expression of vitellogenin band. Thus, FSP levels may be used as an index of maturation in *P. merguiensis* . A similar trend of vitellogenin expression was also reported in the crab *Cancer antennarius* (Spaziani, 1988) and in shrimps *P. indicus* (Laxmilatha, 1991), P. *vannamei* (Quankenbush, 1989a, b) and *Pandalus kessleri* (Quinitio *et al.*, 1989). Several studies demonstrated that vitellogenin is sequestered by oocytes during secondary vitellogenesis and constitutes a main precursor of proteinaceous yolk. Thus, haemolymph acts as a conduit for the ovarian polypeptides during ovarian maturation. In fully mature stage, vitellogenin is absorbed by the developing oocytes as lipovitellin, thereby resulting in altered pattern of haemolymph vitellogenin (FSP). FSP levels in spent females of *P. merguiensis* were higher than in previtellogenic females. Since some females were observed to retain a few maturing oocytes after spawning, the persistence of vitellogenin in the haemolymph of spent females may reflect resorption of residual unexpelled oocytes. However, the high FSP levels in spent females may also indicate that the next vitellogenesis is underway, based on the hypothesis that the source of vitellogenin is outside the ovary and is transported there through the haemolymph (Meusy and Payen, 1988). Similar results were

reported in *P. indicus* (Laxmilatha, 1991), *P. japonicus* (Jasmani *et al.*, 2000) and in the fairy shrimp *Streptocephalus dichotomus* (Munuswamy and Subramoniam, 1987). This suggests the possibility of a feedback mechanism that regulates the synthesis and release of vitellogenin into the haemolymph as well as its uptake in the oocytes. Thus vitellogenin concentration in haemolymph may be used as an indirect indicator of regulatory hormone activities.

Vitellin is the major protein component of the mature ovary. Vitellin from the mature ovaries of *P. merguiensis* was dark-green in colour, similar to those of other penaeids (Quinitio *et al.*, 1990; Qiu *et al.*, 1997). Electrophoretic analysis of ovarian samples revealed the presence of two forms of vitellin (Vn1 and Vn2) in native PAGE (300 and 550kDa). Similar findings were also observed in *P. vannamei* (Quackenbush, 1989a, b), *P. semisulcatus* (Shafir *et al.*, 1992), *P. monodon* (Chang *et al.*, 1994) and *P. chinensis* (Chang and Jeng, 1995a, b). Vn1 was the main vitellin as compared to the expression of Vn2 as shown in the results of native PAGE. Vn2 was only a faint narrow band below Vn1 in native-PAGE. Different molecular weights were clearly shown in two forms of vitellins. Subsequent characterisation of these proteins showed Vn1 and Vn2 to be lipo-glyco- calcium binding and glycoproteins respectively, in *P. merguiensis*.

Different forms of vitellins were reported in penaeids (Table 6). Only one intact form of vitellin was detected in *P. japonicus* (Kawazoe *et al.*, 2000), *P. monodon* (Chang *et al.*, 1993a), *Pandalus kessleri* (Quinitio *et al.*, 1989) and *Parapenaeus longirostris* (Tom *et al.*, 1987) while two forms of vitellins were purified from *P. chinensis* (Chang *et al.*, 1996), and *Macrobrachium rosenbergii* (Derelle *et al.*, 1986; Lee *et al.*, 1997). Similarly in *P. merguiensis*, two forms of vitellins were identified with apparent molecular weights of 300 and 550kDa. It has been found that lipovitellins of six decapod crustaceans had an average molecular weight of 350kDa ranging from 330 to 370kDa (Wallace *et al.*, 1967). Thus, it appears that

Table 6. Molecular mass of vitellogenin and vitellin subunits reported in six penaeid shrimp species

Species		Molecular Mass		References
		Holoprotein	Subunits	
P. monodon	Vitellogenin	ND	74', 83', 104', 168*	Chen and Chen, 1994
		ND	822, 170*	Chang et al., 1994
		ND	74', 83, 104', 200*	Longyant et al., 2000
	Vitellin	540	74', 83', 90, 104', 168*	Quinitio et al., 1990
		ND	74', 83', 104', 168*	Chen and Chen, 1993
		492	35, 45', 49, 584, 64, 68, 82', 9 1	Chang et al., 1993a
			45', 584, 74', 83', 104'	Longyant et al., 1999
P. chinensis	Vitellogenin	ND	85, 191	Chang and Jeng, 1995a,b
	Vitellin	380	40, 58, 78, 85, 105	Chang et al., 1996
		500	78, 85, 155	
P. semisulcatus	Vitellogenin	ND	80, 120, 200	Lubzens et al., 1997
	Vitellin	283	50, 63, 80, 90	Browdy et al., 1990
		283	86, 95	Tom et al., 1992
		ND	80, 96, 158	Khayat et al., 1994
		ND	80, 120, 200	Lubzens et al., 1997
P. vannamei	Vitellin	289	61, 69	Tom et al., 1992
		ND	76, 95, 97, 103	Quackenbush, 1989a,b
Parapenaeus longirostris	Vitellin	ND	45, 66	Tom et al., 1987
Metapenaeus ensis	Vitellin	350	76, 102	Qiu et al., 1997

ND. Not determined . The superscripts indicated the equivalent vitellin or vitellogenin subunits found by other studies.

the molecular weight of native vitellin in most penaeids was about 300kDa. Vitellins of higher molecular weight have been also reported in other penaeids. In *P. japonicus* (Vazquez-Boucard *et al.*, 1986) and crayfish *Procambarus* species (Fyffe and O'Connor, 1974), the molecular weight of vitellin was reported as 500kDa. Similarly, the molecular weight of vitellin in shrimps *Pandalus kessleri* (Quinitio *et al.*, 1989) and *P. monodon* (Quinitio *et al.*, 1990) was 550 kDa and 560 kDa respectively.

Vitellin in *P. merguiensis* had five major polypeptides, the molecular weights of which were 104, 102, 68, 55 and 44kDa as revealed by 12% SDS-PAGE. In various penaeid shrimps, 2-5 polypeptide subunits were demonstrated in the vitellin (Vazquez-Boucard *et al.*, 1986; Tom *et al.*, 1987; Quinitio *et al.*, 1989, 1990; Chang *et al.*, 1996; Longyant *et al.*, 1999, 2000). Three polypeptide fraction with molecular weight 104, 102 and 68kDa were found commonly present in both vitellogenin and vitellin. This support the relationship that some subunits of ovarian vitellin were possibly and directly derived from haemolymph vitellogenin suggesting haemolymph polypeptides to be precursors of some yolk proteins. It is suggested that after incorporation of vitellogenin into oocytes, it may get partially digested and transformed into separate subunits forming the dominant components of ovarian vitellin. Similar results were reported in *P. chinensis*, where vitellogenin was shown to have two polypeptide subunits (191 and 85kDa) of which 191kDa was partially digested and transformed into vitellin in combination with 85kDa (Chang and Jeng, 1995a, b; Chang *et al.*, 1996). Further studies about the relationship of polypeptide subunits of vitellogenin and vitellin are necessary to clarify their association in *P. merguiensis*.

Both vitellogenin and vitellin isolates from vitellogenic females satisfied many of the properties cited for these proteins in other species of crustaceans. Both appeared only during the period of vitellogenesis, contained lipid and carbohydrate moieties and showed an affinity for calcium as revealed by the different staining capacities (Sudan Black B, PAS and Alizarin Red S

respectively). They had more or less the same mobility and their molecular weights were 550 and 300kDa for vitellin and 350kDa for vitellogenin and were comparable to those reported for many other crustacean species. An increase in the affinity of the female specific protein (vitellogenin) and vitellin to Alizarin Red S indicates their affinity to calcium. The high calcium content of vitellogenin promotes the solubility of this protein and much of the vitellogenin-bound calcium may be incorporated into the oocytes to meet the needs during embryonic development. Thus haemolymph serves as a transporting medium for calcium to the developing oocytes. The protein fraction of vitellin showed similar staining reactions as that of vitellogenin. A pronounced increase in the staining capacity to Alizarin Red S was observed in the third stage of maturity in the haemolymph and in mature ovaries (Stage IV) followed by a decrease in the subsequent stages. A similar increase in calcium in the haemolymph was reported in *P. indicus* (Laxmilatha, 1991) during the reproductive cycles, which increased from the immature stage to the mature stage. An increase in the calcium content in both the serum and ovaries are reported during vitellogenesis in several teleosts (Oguri and Takada, 1967; Peterson and Shehadeh, 1971; Hara *et al.*, 1980; Tinsley, 1985; Wallace, 1985; Gopalakrishnan, 1991). The pronounced rise in the concentration of calcium in stage III, could be associated with the presence of appreciable quantities of vitellogenin at this stage.

In crustaceans, the origin of vitellogenin in the haemolymph is still controversial. The site of synthesis of yolk protein or their possible precursor has not yet been clarified in crustaceans. In amphibians (Wallace and Bergink, 1974) and teleosts (Bun and Idler, 1983) the synthesis of this lipoprotein occurs at extra ovarian sites like hepatocytes from which it is subsequently transported to the ovary during vitellogenesis. In several marine crustaceans the origin of yolk is reported to be exclusively intra oocytically (auto synthesis) or transported from extra oocytic source (heterosynthesis) and the origin varies from species to species. Possible sites of vitellogenin synthesis in crustaceans include ovary, hepatopancreas and adipose tissue (Wolin *et al.*, 1973; Lui and O'Connor, 1976;

Croisille and Junera, 1980; Picaud, 1980; Souty and Picaud, 1981; Meusy *et al.*, 1983; Eastman-Reks and Fingerman, 1985; Paulus and Laufer, 1987; Quackenbush, 1989a, b; Fainzilber *et al.*, 1992). However, several reports show that vitellin is synthesised in the ovary. Thus, the precise site(s) of vitellogenin synthesis in penaeid shrimp is hitherto unknown. Given the apparent contradictions in the evidence for yolk production in crustacean oocytes, an in-depth assessment of the proportion of yolk that is synthesised within the oocyte and the proportion that is imported from the haemolymph by micropinocytosis is needed. Nevertheless, the total quantity of crustacean yolk protein accumulated is a net result of both intraoocytic and extraoocytic syntheses seem certain; however, the relative contribution of each needs to be better defined in *P. merguiensis* also.

In conclusion the present study conducted to partially purify and isolate vitellogenin from the haemolymph and vitellin from the ovaries from vitellogenic females in *P. merguiensis* revealed the following facts:

1. The discovery of vitellogenin (molecular weights of 350kDa) in the serum of mature female and two corresponding vitellin (molecular weights of 300 and 550kDa) in the ovary during vitellogenesis.

2. The vitellogenin and vitellin thus isolated have been characterised as glyco-lipoproteins based on their staining capacity.

3. Both have more or less same electrophoretic mobility.

4. The present study revealed calcium-binding capacity for both vitellin and vitellogenin based on their staining capacity to Alizarin Red S.

5. Vitellogenin may be a possible precursor of egg yolk protein.

Chapter 3

EXPERIMENTS ON INDUCED MATURATION IN *PENAEUS MERGUIENSIS*

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Shrimps dominate the global production of farmed crustaceans (FAO, 1997). Despite the perceived potential from increased production in aquaculture, the sustainability of shrimp farming is currently threatened by low production efficiency and vulnerability of farmed stocks to diseases (Rosenberry, 1997). One approach to overcome these threats is to develop domesticated seeds for selected traits. However, this approach is currently hampered by the reliance of the industry on wild broodstocks and lack of information on the response of shrimps to induce maturation treatments.

The key to successful domestication of candidate species in culture activities lies in controlled and enhanced reproduction of broodstock. Shrimp farming industry has generally been slow to domesticate species and commence selective breeding programmes compared to terrestrial farming. Dearth of knowledge on the basic physiology of gonadal development of penaeid shrimps and the inability to regulate the production of viable eggs hampers their aquaculture. But large-scale expansion of shrimp farming activities all over the world has resulted in their controlled breeding under captivity a necessity. Production of predictable abundant supply of offspring of known heritage on a precise time schedule and successful rearing through larval stages are the critical factors in this industry. Many advances have been made in the development of technology for the captive maturation and reproduction of penaeid shrimp (Primavera, 1985; Bray and Lawrence, 1992; Browdy, 1992). Nevertheless, when available, hatchery managers often prefer nauplii from gravid females collected from natural spawning grounds (Kawahigashi, 1992) as females, which mature in the wild, are thought to produce high numbers of superior nauplii compared to captive matured stocks. But reliance on wild broodstocks for seeds have proven limited and unreliable in supporting this rapidly expanding industry due to the

uncertainty of their availability at appropriate time in requisite quantities. This also precludes the opportunity to enhance production through controlling the spread of disease or selective breeding for commercially beneficial trials such as improved survival and growth. If broodstock diet and conditions in a maturation system are well maintained, quality of spawns of captive matured females may be as high as that of wild matured broodstock (Browdy, 1998). Thus broodstock management assumes great importance and of late intensive research effort has been directed towards the controlled husbandry of marine penaeid shrimp.

The current method used world-wide for commercial development of captive broodstock of penaeid shrimps relies almost exclusively on unilateral eyestalk ablation following the pioneering work of Panouse (1943) in *Leander serratus* in conjunction with water quality management (Browdy, 1992). Reproduction in crustaceans has been hypothesised to be controlled by dual endocrine factors, one inhibitory (gonad inhibitory hormone, GIH) and other stimulatory (gonad stimulatory hormone, GSH), both originating from the neuroendocrine centres such as the eyestalk ganglia and the brain / thoracic ganglia respectively (Adiyodi and Adiyodi, 1970). In majority of malacostracan crustaceans, eyestalk is the pivotal organ for housing various neuropeptides. Unilateral eyestalk ablation has been found to be adequate to stimulate ovarian maturation and spawning, indicating that lowering the titre of GIH below a certain threshold level by single eyestalk ablation is sufficient to stop its inhibitory effect on the stimulatory neuropeptides or other putative gonadotropic hormonal source. Similar results have been subsequently made for a number of species. GIH has been partially purified by Bomirski and Kleke (1974) and Quackenbush and Hernkind (1981). This hormone apparently occurs in nature in shrimps during the non-breeding season and is absent or present in low level during breeding season (Bomirski and Kleke, 1974; Kulkarni and Nagabhushanam, 1980). The threshold level of GIH to inhibit or restrain the gonadal development has not yet been determined for any shrimp species (Subramoniam, 1999). Eyestalk ablated shrimps respond to their operation with a rapid and unstoppable gonadal

development. Even species, which develop ovary and spawn in captivity, eyestalk ablation reduces the interbreeding time significantly, thus augmenting total egg production in a given time. In addition, eyestalk ablation is also helpful in extending the period of annual breeding cycle in the shrimps. In many species of crustaceans, the response of ovary to the absence of GIH as a result of eyestalk ablation varies with respect to the season of the year or the stage of annual reproductive cycle (Eastman-Reks and Fingerman, 1984). The variation in response of the ovary to GIH removal in these crustaceans suggests the presence of a second hormone, ovary stimulating hormone (OSH/ GSH), which in turn is released only when ovarian growth is to occur. Thus the bihormonal theory of ovarian regulation is based on the principle that ovarian growth is balanced through the antagonistic action of the two hormones - OIH and OSH (Adiyodi and Adiyodi, 1970).

The efficacy of eyestalk ablation for increasing spawning rate has been reported in several penaeids and is now practised in shrimp hatcheries worldwide. The technique has been adopted for inducing maturation of captive stock with varying degrees of success by pioneers and over 20 species of commercially important shrimp species have responded positively to eyestalk ablation (Caillouet, 1972; Arnstein and Beard, 1975; Alikunhi *et al.*, 1975; Aquacop, 1975 and 1977a, b; Duronslet *et al.*, 1975; Wear and Santiago, 1976; Santiago, 1977; Halder, 1978; Primavera, 1978; Brown *et al.*, 1979; Lumare, 1979; Kelemec and Smith, 1980; Lawrence *et al.*, 1980; Chamberlain and Lawrence, 1981; Emmerson, 1983; Charniaux–Cotton,1985; Trujillo and Primavera, 1986; Quackenbush, 1986; Choy, 1987; Fingerman,1987; Makinouchi and Primavera, 1987; Charniaux–Cotton and Payen, 1988; Meusy and Payen, 1988; Van-Herp,1988; Hossain *et al.*, 1990; Rothlisberg *et al.* ,1991; Marian and Murugadass, 1991; Tan-Fermin, 1991; Wyban and Sweeney,1991; Bray and Lawrence,1992; Browdy, 1992, 1998; Cardona and Capo, 1992; Primavera and Caballero, 1992; Robertson *et al.*, 1993; Yano, 1993; Mohamed and Diwan, 1994; Makinouchi and Hirata, 1995; Ramos *et al.* , 1995; Jetani *et al.* , 1996;

Lumare *et al.*, 1996; Palacios *et al.*, 1999). This indicates that unilateral eyestalk ablation greatly enhances gonadal maturation in most of the penaeids like *P. merguiensis, P. indicus, P. stylirostris, P. vannamei* and *P. setiferus* and it is a prerequisite for hardy penaeids like *P. monodon* (Aquacop, 1979).

Eyestalk ablation suspends the controls on a large number of body functions, so that females so treated divert all their energies into ever more frequent bouts of egg production, leading to a loss in egg quality and eventual death. Many commercial hatcheries prefer eyestalk ablation to induce maturation in penaeid shrimps inspite of the associated disadvantages of increased mortality, disruption of the shrimp's endocrine system and decreased larval viability with repeated spawnings. This is mainly due to the predictable peaks in maturation and spawning with eyestalk ablation, which facilitates the setting up of production schedules, in contrast to scattered spawns from unablated females. For production purposes, this predictability of availability of spawners and number of nauplii compensates for the trend of decreased fecundity and hatching rates with successive spawns from ablated broodstock. The effect of eyestalk ablation on fecundity and egg quality is less clear. Reduced fecundity and hatching rate were reported for ablated *Penaeus indicus* (Emmerson, 1980) and *P. monodon* (Primavera and Posadas, 1981). Browdy and Samocha (1985a, b) found lower fecundity per spawn, but greater overall egg production for ablated *P. semisulcatus*; however no difference in hatching rate or larval survival between ablated and non-ablated females was reported. Lawrence *et al.* (1980) could not find any significant distinction between ovarian maturation with or without eyestalk ablation in penaeid shrimps. Overall larval performance and survival was reported to be considerably high in recently ablated spawners (15 days) and decreased in spawners 45 and 75 days after ablation (Palacios *et al.*, 1999). Thus, shrimps provided with quality diets and suitable environment, eyestalk ablation *per se* does induce and accelerate ovarian growth, leading to normal spawning and healthy offspring in the pond reared as well as wild females.

The development of an alternative technique that would allow the production of high quality eggs over time without the side effects of eyestalk ablation would be of immense benefit to the penaeid shrimp culture industry. Various alternatives to eyestalk ablation have been tried based on accumulated knowledge about crustacean endocrinology. Under consideration are the use of crustacean ganglionic extracts prepared from vitellogenic females (Yano, 1992,1993), juvenile hormone-like compounds (Laufer *et al.*, 1987; Tsukimura and Kamemoto, 1991; Wilder *et al.*, 1995), vertebrate hormones (Browdy, 1992) and neurotransmitters (Van Herp, 1988; Kulkarni *et al.*, 1991; Richardson *et al.*, 1991).

Literature on the hormonal control of crustacean reproduction has been extensively reviewed by Adiyodi (1985), Payen (1986), Meusy and Payen (1988), Charniaux-Cotton and Payen (1988), Van Herp and Soyez (1997), Charmantier *et al* (1997) and De Kleijn and Van- Herp (1998). Otsu and Hanoaka (1951) first postulated that a factor produced by the central nervous system (CNS) promotes ovarian growth. Since then several investigators have reported that implants of thoracic ganglia from mature females stimulate precocious vitellogenesis *in vivo* in immature females (Gomez, 1965; Oyama, 1968; Hinsch and Bennett, 1979; Eastman-Reks and Fingerman, 1984; Takayanagi *et al.*, 1986; Yano *et al.*, 1988). This principle was tested on *P. vannamei* by implanting thoracic ganglia from maturing *Homarus americanus* (Yano *et al.*, 1988). Accumulation of yolk granules in oocytes stimulated by thoracic and supraoesophageal ganglion implants from the mature females in immature females, has been reported in several crustaceans like in crab *Potamon dehaani* (Otsu, 1960), *Paratelphusa hydrodromous* (Gomez, 1965), *Thalamitta crenata* (Oyama, 1968), *Libinia emarginata* (Hinsch and Bennett, 1979) and *Uca pugilator* (Eastman- Reks and Fingerman, 1984), and in shrimps *P. japonicus* (Yano and Chinzei, 1987; Yano, 1992), *P. vannamei* (Yano *et al.*, 1988; Tsukimura and Kamemoto, 1991), *P. monodon* (Har, 1991), *Parapenaeopsis hardwickii* (Kulkarni *et al.*, 1981), *P. stylifera* (Nagabhushanam and Joshi, 1986) and in *Paratya compressa* (Takayanagi *et al.*, 1986). The stimulatory effect of these ganglionic

administrations has been attributed to some peptides produced in brain, which function as gonad stimulatory hormone releasing factor (GSH-RF), so as to act on the thoracic ganglia to release the GSH. While thoracic ganglionic extracts prepared from vitellogenic females had gonad stimulatory activity, extracts prepared from previtellogenic females had no activity in *P. japonicus*, clearly suggesting the secretion of GSH only in vitellogenic female shrimps (Yano and Chinzei, 1987).

Administration of micro-quantities of purified hormone is a recently developed technique to accelerate gonad maturation in crustaceans on which intensive research is still being carried out. Vitellogenesis and its regulation by hormones have been described for many vertebrate species, but information on the same in invertebrates is scanty. There is now substantial evidence to suggest that steroid hormones may play an important role in regulation of vitellogenesis. Steroids are biologically active in crustaceans and they have been found in the ovarian tissues of *P. monodon* at different stages of vitellogenesis (Fairs *et al.*, 1990). Several decapod crustaceans have been reported to have the ability to synthesise vertebrate-type steroid hormones. Such substances in invertebrates are novel and have great evolutionary significance. The exact physiological and biochemical role of such steroids in crustaceans are yet to be fully established. The evidence for the presence of steroids in crustacean tissues, the ability of steroids to stimulate vitellogenesis and the identification of neurosecretion regulating the vitellogenic process suggest that ovarian development and oocyte maturation may be regulated by a system similar to that operating in teleost fish (Wallace and Selman, 1981) and amphibia (Tata and Smith, 1979). In these animals both vitellogenesis and oocyte maturation are known to be regulated by steroid hormones.

Vaca and Alfaro (2000) proposed that steroid hormones, similar to that in teleost fish and amphibia, might regulate ovarian development and oocyte maturation in crustaceans. Effect of such hormones on ovarian maturation has

been examined in various crustaceans such as crabs *Thalamitta crenata* (Oyama, 1968), fresh water crab *Oziotelphusa senex senex* (Reddy and Ramamurthi, 1998) and in shrimps *Crangon crangon* (Bomirski and Klek-Kawinska, 1976), *P. japonicus* (Yano, 1987; Wilder and Aida, 1995), *Parapenaeopsis hardwickii* (Kulkarni *et al.*, 1979), and in freshwater prawn *Macrobrachium* species (Sarojini *et al.*, 1985, 1986; Wilder *et al.*, 1995). Progesterone, a vertebrate-type steroid hormone, has been detected in several crustaceans and its effect on gonad maturation has been largely reported. Kulkarni *et al.* (1979) showed that progesterone stimulates oogenesis in *P. hardwickii*. Similar maturation stimulatory effects of progesterone exist in several crustacean species such as in Greasy back shrimp *Metapenaeus ensis* (Yano, 1985); *P. merguiensis* (Chan and Lim, 1988); *P. japonicus* (Yano, 1987) and in *P. monodon* (Joseph, 1996). Vaca and Alfaro (2000) found 17α-hydroxyprogesterone to significantly increase *P. vannamei* oocyte diameter, *in vitro*. Young *et al.* (1992) studied the metabolism of progesterone in decapod crustaceans.

A completely new approach to induce gonadal maturation and spawning in crustaceans relies on the role neurotransmitters play in regulating gonadal maturation as defined by Fingerman (1997). There is continuing and increasing interest in identifying the role of neurotransmitters in inducing the release of neurohormones that regulate the reproductive activities in both vertebrates (Crim *et al.*, 1984) and invertebrates (Fingerman, 1987). Several neurotransmitters have been identified as affecting the release of neurohormones in crustaceans. 5-hydroxytryptamine (5-HT), found in the central nervous system of crustaceans, is a biogenic amine functioning as a neurotransmitter/ neuromodulator / neurohormone in a wide variety of species including crustaceans (Fingerman *et al.*, 1974; Elofsson *et al.*, 1982, 1983; Kulkarni and Fingerman, 1992). In Crustacea some of these amines also subserve the function of neuroregulators to control the release of crustacean neurohormones by stimulating the release of several eyestalk neuropeptides. 5-HT has been found to stimulate

the release of crustacean moult inhibiting hormone (Fingerman, 1997). Other functions reported to be played by 5-HT include migration of the proximal retinal pigment, pericardial organ neurohormone, stomatogastric ganglion neuromodulator or neurohormone, behavioural responses, osmoregulation and mechanoreception (Fingerman, 1997). 5-HT like immunoreactivity was reported in the axons of the lamina ganglionaris of the eyestalk of *Procambarus clarkii* (Arechiga *et al.*, 1990) and in the brain of the crayfishes *Pacifastacus leniusculus* (Elofsson, 1983).

Some authors have reported 5-HT to play a role in inducing ovarian maturation. In marine animals, at least in some bivalves, it apparently regulates spawning and oocyte maturation. 5-HT has been routinely used for induced spawning in many molluscs. The role of 5-HT on the ovarian maturation in crustaceans was indicated by its stimulatory effect on ovarian development in fiddler crab *Uca pugilator* (Richardson *et al.*, 1991; Kulkarni and Fingerman, 1992; *Procambarus clarkii* (Kulkarni *et al.*, 1992; Sarojini *et al.*, 1995), *P. vannamei* (Vaca and Alfaro, 2000), *P. penicillatus* (Oliviera and Correa, 1999) and *Paratelphusa hydrodromous* (Ragunathan and Arivazhagan, 1999). These findings are consistent with the hypothesis that 5-HT exerts its stimulatory effect on the ovaries of crustaceans indirectly by triggering release of gonad stimulating hormone (GSH) from the brain and thoracic ganglion.

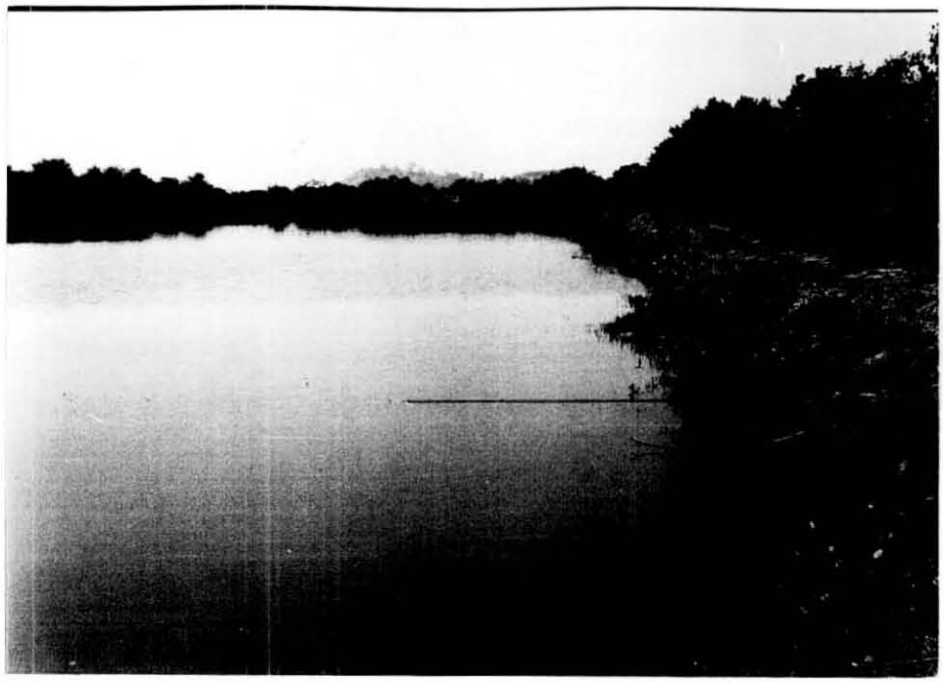
Although the mode of action of these exogenous substances in the crustacean egg production is not properly understood, there seems to be tremendous application potential in using these to stimulate gonadal maturation in aquaculture species and the possibility of introducing new strategies in induced ovarian maturation in aquaculture. The present study was conducted with an aim to develop captive broodstock of *P. merguiensis* by means of eyestalk ablation and administration of exogenous substances such as ganglionic extracts, 17α-hydroxyprogesterone and 5-hydroxytryptamine.

MATERIALS AND METHODS

Adult females of *P. merguiensis* (120-150 mm in total length and 15-20 gm in weight) with undeveloped ovaries and in the intermoult stage were selected for experiments to assess the effect of eyestalk ablation under different environmental conditions, administration of ganglionic extracts, vertebrate type steroid hormone (17α-hydroxyprogesterone) and neurotransmitter (5-hydroxytryptamine) on ovarian maturation. Animals were collected both from traditional culture ponds and from open sea for the experiments. Healthy animals collected were transported in oxygenated seawater to the laboratory in polythene bags. Continuous aeration was provided during the transportation. Upon their arrival to the laboratory, they were disinfected with 50mg/l formalin for 1-2 minutes and transferred to aerated plastic pools containing seawater (salinity 25 ± 2‰; temperature : 29 ± 2^{0}C) and maintained at normal day-night illumination. They were fed with fresh clams and squids *ad libitum* once daily at the rate of 10% of their body weight.

After an initial acclimatisation period of 48 hours, sexually immature females were subjected to different treatments and introduced into the experimental unit set up in the bay. At the termination of the experiment, gonadal index, an indicator of reproductive activity of marine invertebrates, which forecasts the development of the gonads was determined in all the treatments. The responses of *P. merguiensis* were also analysed histologically and electrophoretically. The data were also subjected to statistical analyses.

PLATE 13. Brackishwater ponds from where samples were collected



Experimental unit

The experiments were carried out in velon-screen net cages (fish breeding hapa of 2x1x1 m; mesh size - 225 mesh /sq. inch) (two numbers) installed in Karwar Bay about 500 m away from the shore line and at a depth of two metres (Plate 14). The net cages (hapa) had a provision for opening at the top. They were held in position by fastening to casuarina poles driven to the bottom and were provided with suitable anchors at the lower corner of each net. They were given an outer secondary covering of bigger mesh size (9 mesh per sq. inch) to avoid damage by predators, especially crabs. The units were set out during low tide so that they remained completely immersed even at the lowest low tide and were 1 foot off the bottom to avoid climbing of crabs. PVC tubes of 20cm length and 4cm diameter, with adequate anchoring weights, were introduced as possible artificial hideouts for shrimps.

1) Unilateral eyestalk ablation

Endocrine manipulation has so far been synonymous with unilateral eyestalk ablation. Several important species of penaeid shrimps have been induced to mature and spawn in captivity by eyestalk ablation technique, but the percentage of animals getting matured by this technique varies greatly. In the present study a series of eyestalk ablation experiments were conducted at different environments to assess their effect on pond reared as well as wild *P. merguiensis*.

In the first series of eyestalk ablation experiment ten adult *P. merguiensis* with undeveloped ovaries collected from the brackishwater shrimp ponds (Kali estuary) were subjected to unilateral eyestalk ablation and introduced into the experimental units established in the bay (Temperature: 29 $\pm$ 1^{0}C; Salinity: 27- 33‰). The females were observed thrice during the experimental period for ovarian maturation. The study was carried out for a period of 10 days.

PLATE 14. Setting up of experimental units in Karwar Bay for maturation studies

Observations were made on the third, fifth and eighth day. At the termination of the experiment the shrimps were examined for the gonadal conditions based on visual observation of maturation through the exoskeleton and also by histological techniques.

In the second series, shrimps from filtration ponds ranging from 80-110 mm in total length (18-20mm carapace length) with immature ovaries were subjected to eyestalk ablation and introduced into the experimental units. As in the previous experiment, the females were observed thrice during the experimental period for ovarian maturation. The hydrobiological conditions of the bay were similar to that of the previous experiment (Temperature : 29 ± 1^{0}C; Salinity : 27- 35‰).

The effect of season on the reproductive performance of broodstock of penaeids has not been well documented. Hence in the third series of experiment, an attempt was made to assess the effect of eyestalk ablation on ovarian maturation in *P. merguiensis* during the colder months in nature. Five adult female shrimps (size range 120-150 mm) with immature ovaries were subjected to unilateral eyestalk ablation during December month and observed for a period of 10 days. The temperature during the daytime varied between 27^0 C and 29^o C and during night, the temperature fell to 18^{0}C to 22^0 C. At the termination of the experiment both the ablated and control animals were examined critically for the gonadal conditions.

Animals collected from the same area, with similar biological characters and reared under similar experimental conditions, but without eyestalk ablation served as controls in the experimental trials. Males were also introduced into the units in all the experiments tried in the ratio of two females to one male. The total experimental duration was 10 days. The development of gonad was determined externally by holding each female against a powerful light and observing the state of ovary through the cephalothorax and abdomen. Fully matured animals were

transferred to spawning tanks of 100lt capacity. Egg counts of females were taken in the morning in the following day. Animals, which did not spawn, were sacrificed for assessment of gonadal development after 10 days of the experiment. Morphological features such as colour, nature and development of ovarian lobes were noted and small pieces of ovarian tissues were fixed in Bouin's fixative for histological evaluation. Staging of gonadal development was done according to Rao (1967).

1) Ganglionic extract treatment

The second series of experiment was designed to study the effect of cerebral and thoracic ganglionic extracts on ovarian maturation in *P. merguiensis*. The experimental unit and site of the experiment were similar as described in the previous experiment. The experiments were conducted during the warmer months (February – March). To preclude any variation in the results, healthy shrimps of uniform size (120-140 mm total length) and gonadal development were introduced into the hapa along with males in the ratio of one male to two females. Animals in the test groups (5 number in each) were administered a single injection of 0.2ml of brain/ thoracic ganglionic extract obtained from freshly dissected female shrimp in the vitellogenic stage. An aqueous extract of brain and thoracic ganglion of one shrimp in the vitellogenic stage was taken as one CNS (Central nervous system) equivalent. The ganglion were macerated in normal saline (0.85% NaCl) and centrifuged at 3000g for 10 minutes. The supernatant thus obtained was used for injection. Five females injected with 0.2ml of normal saline (NaCl 0.85%) formed the control group. Shrimps were injected through the first abdominal somite using a hypodermic syringe only once to avoid excessive handling stress.

Shrimps were monitored for signs of gonad development. After 10 days, both the controls and the treated females were sacrificed and GSI determined. Ovarian samples were removed for histological analysis. Haemolymph

samples were also collected from the shrimps for electrophoretic analyses to study the appearance of female specific protein associated with ovarian maturation.

2) Steroid Hormone treatment

Crustaceans are reported to respond to various vertebrate- type steroid hormones. In the third series of experiments immature females of *P. merguiensis* were injected once with 17α- hydroxyprogesterone (Schering A. G, Germany) @ 0.1μg/gm body weight to study its effect on the morphological and histological structure of the ovaries. Pure ethanol was used as the carrier vehicle of the hormone in the shrimps. The hormone was dissolved in the carrier solution immediately prior to use. Females (5 number) injected with 100μl of ethanol formed the control group.

After a period of 10 days both the treated and control groups were removed from the hapa and their GSI calculated. Haemolymph and ovarian samples were also collected and preserved for electrophoretic and histological analyses.

3) Neurotransmitter treatment

Neurotransmitter 5-HT was administered intramuscularly to five females with immature ovaries in the fourth series of experiments to study its impact on ovarian maturation in *P. merguiensis*. The females received three injections of 5-HT creatinine sulfate (Sigma, St. Louis, MO, USA) on the first, fifth and tenth day at the rate of 15 μg/g BW. Five females injected with ethanol (100μl) formed the control. The treated females were introduced into fish breeding hapa set out in the bay along with control females and males (in a ratio of one male to two females). Shrimps were monitored for signs of gonad development. The females treated with 5 - hydroxytryptamine and their controls were sacrificed on the fifteenth day. Small tissue samples were removed from the middle lobe of the

ovary and fixed in Bouin's solution and prepared for histological observation. Haemolymph samples from the treated females were also subjected to electrophoretic analyses.

RESULTS

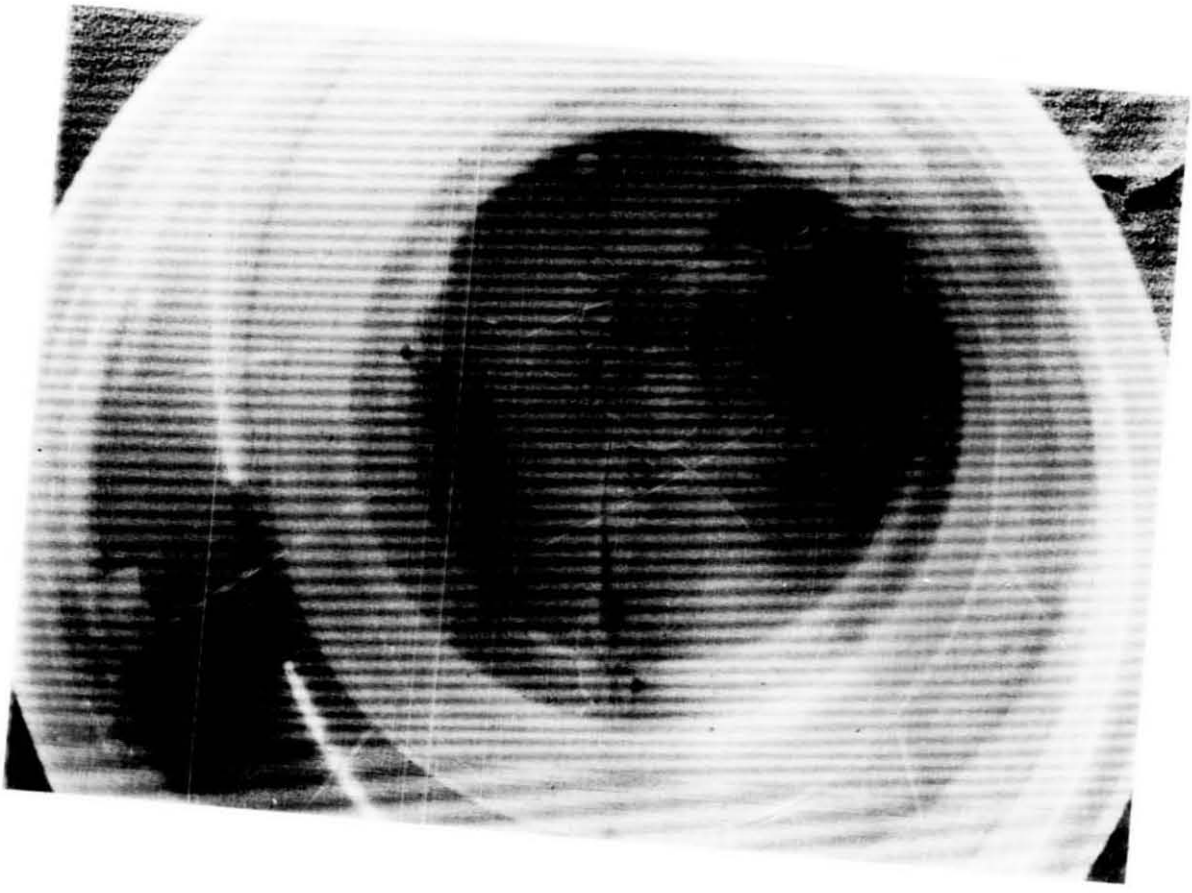
In crustaceans, most of the physiological processes, including reproduction and maturation, are controlled by neuroendocrine system. The present experiments were conducted to study the effect of eyestalk ablation, administration of ganglionic extracts, hormone and neurotransmitter on gonadal maturation of marine and farm raised *P. merguiensis*.

1) Unilateral eyestalk ablation experiments

a) *Influence of unilateral eyestalk ablation on gonadal maturation of estuarine farm raised P. merguiensis under marine conditions.*

A significant increase in the GSI and oocyte diameter was exhibited in all the unilaterally eyestalk ablated females in the present study. On the third day of observation, among the eyestalk ablated females, only one female was found to reach the second stage of maturity while others did not show any signs of ovarian development (based on external visual observation). However, 50% of them demonstrated well-defined ovarian development within 5 days with 4 reaching the second stage and one in the advanced third stage of development (Plate 15). On the eighth day, the females formerly seen in the advanced stages of maturation were found to have spawned in their natural environment. However, two more females were found to show advance development in the ovarian maturation (Stage III). They were assumed to be the late responders to the ablation technique. One of them were moved to another hapa of finer mesh (mesh size of 300 microns) set out adjacent to the former hapa while the other was shifted to the

PLATE 15. Illustration of ovarian maturation in unilaterally ablated females maintained in the bay waters

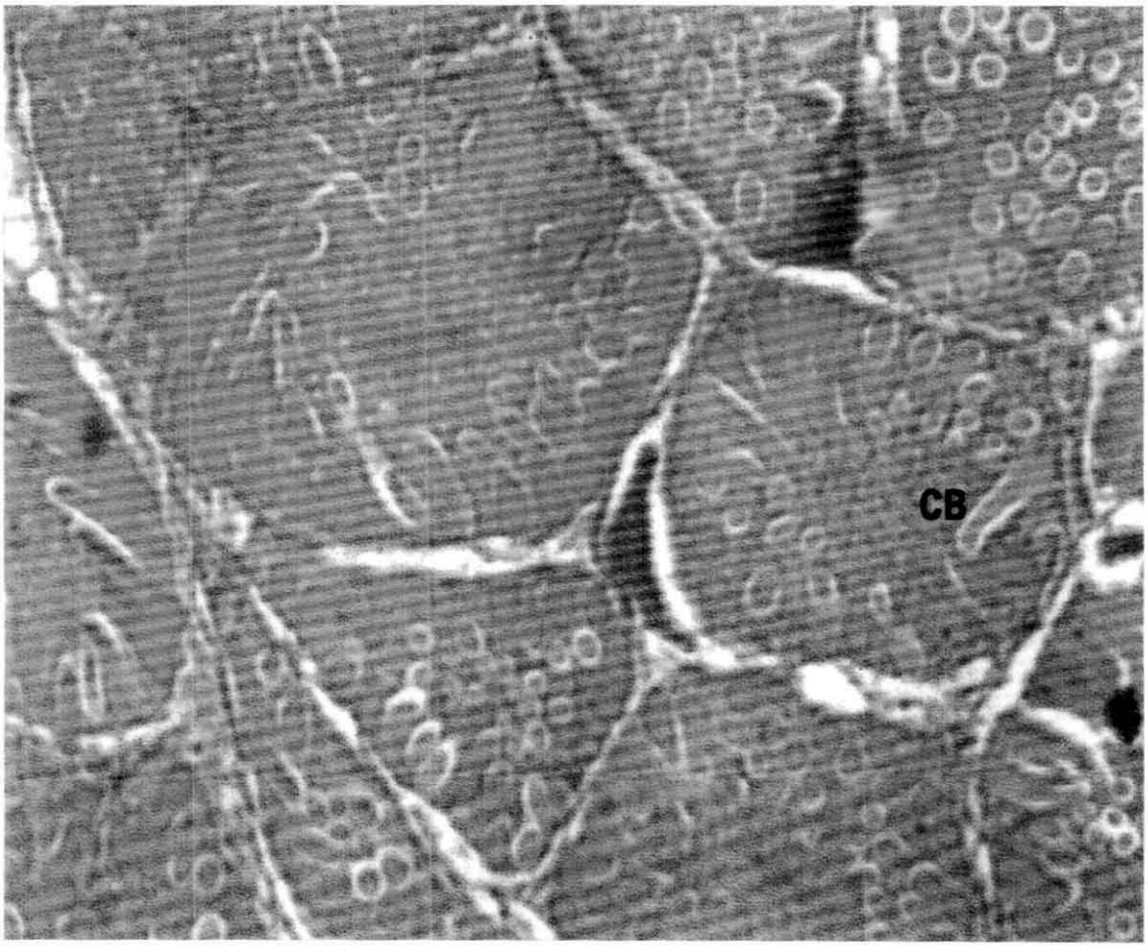


laboratory in oxygenated polythene bag. The shrimp maintained in laboratory spawned the next day giving rise to nauplii on the ninth day. On the 10th day of observation, the spawner retained in hapa was also found to be in the spent conditions indicating that the spawner had spawned on the 9th night similar to the spawner maintained in the laboratory. All the animals were sacrificed on the 10th day and ovarian samples were preserved for histological analyses. No mortality was observed in both eyestalk ablation and control groups during the experimental period.

Ovaries of ablated females in late vitellogenic stage, in the present experiment, were visible as a thick, solid, linear band, which expanded at the posterior thoracic and abdominal segments through the dorsal exoskeleton. The ovaries appeared greenish yellow to olive green in colour. Light microscopic studies revealed that these yolky oocytes in vitellogenic ovary were equipped with the characteristic cortical rods (Plate 16). At the termination of the experiment no apparent ovarian development or spawning was observed in the control animals. The ovaries of these animals appeared translucent and /or opaque with a white to cream colour and smooth texture. The undeveloped ovaries were evidenced by the low mean GSI value 0.25- 0.4. Light microscopic observations revealed that the ovarian lobes were packed with developing oogonial cells and primary oocytes with a prominent nucleus and chromatin material. The oocyte diameter varied between 12-45μ in control. The haematoxylin stained cytoplasm did not indicate any signs of yolk formation. There was no mortality during the experiment and the feeding and swimming behaviour of the animals were normal.

About 1,80,000 eggs were obtained from single spawn by the ablated females under laboratory conditions. The eggs hatched out the following day (87%) indicating that the eggs were of good quality. The shortest period observed from ablation to the onset of maturation was 3 days and from ablation to spawning was 6 days.

PLATE 16. Ovaries of unilaterally ablated females revealing ripe oocytes with cortical bodies (CB – Cortical Bodies)



b) *Eyestalk ablation (ESA) of smaller sized P. merguiensis*

In the second series of eyestalk ablation experiments, females smaller than the minimum size of first maturity were subjected to eyestalk ablation. On termination of the experiment, both control and the experimental groups showed no signs of ovarian development. Ovary was not visible through the dorsal exoskeleton. The eyestalk ablated females were observed to be still in the first or previtellogenic stage of gonadal development at the end of the experiment. Ovarian lobes were translucent and smaller in diameter than that of the underlying gut. Histologically, the ovarian lobes were packed with the developing oogonia and previtellogenic oocytes, which are characteristic features of immature ovaries. Eyestalk ablation did not induce any progressive change in ovarian maturation during the experiment. The shrimps exhibited normal feeding and swimming behaviour.

c) *Influence of unilateral eyestalk ablation on P. merguiensis during the colder months*

Unlike the similar experiment conducted earlier during the warmer months, no obvious changes were observed in the ovaries in the third day both in the eyestalk ablated and control females. However, on the seventh day signs of ovarian maturation were visible in all the eyestalk ablated females, while no signs of ovarian maturation were observed in the control females. The ovaries of the ablated females appeared greenish yellow to olive green in colour, but were restricted only to the anterior end. Thus the ovaries showed a minimum growth. Hundred percent survival was observed during the experiment. No further development was observed till the termination of the experiment on the 10^{th} day. The animals were sacrificed and the GSI was calculated (1.2- 2). Light microscopic studies revealed that these yolky oocytes were in early vitellogenic stage. The average oocyte diameter was 90µ. Thus ovarian maturation in

P. merguiensis as evident from the results of the present experiment was slow in eyestalk ablated females during the colder months. Hence, it is evident that higher temperature of warmer period enhances the gonadal development.

2) Ganglionic extract treatment

Females injected with brain and thoracic ganglionic extracts exhibited an increase in the GSI compared to the controls. The average GSI of the control was 0.357 and those of the brain and thoracic ganglion treated groups were 0.9352 and 0.525 respectively. The ovarian indices and oocyte diameter of the cerebral ganglionic extract treated group had increased significantly compared to that in females injected with thoracic ganglion extract and control. Histological observations in the controls showed only previtellogenic oocytes with average oocyte diameter of 30µ. Similarly in the thoracic ganglion treated group, about 55% of the oocytes were in previtellogenic stage with ova diameter ranging between 56 - 112µ, while the rest of the oocytes (nearly 45%) were in the early vitellogenic stage of maturation with ova diameter ranging between 64 - 152µ. But in the cerebral ganglion treated group, more advancement in maturation was visible with the oocytes (nearly 68% of the oocytes in the early vitellogenic stage) becoming less basophilic and cytoplasm becoming granular (Plate 18a). The ova diameter of the females receiving cerebral ganglionic extract ranged between 72- 188µ.

Electrophoretic analyses of the haemolymph samples from females treated with cerebral ganglionic extract revealed that the expression of female specific protein (FSP) in these females were comparable to that of early maturing females. The expression of FSP was faintly visible in the females receiving thoracic ganglionic extracts.

One way analysis of variances of the GSI of females treated with thoracic and brain extracts showed that the ovarian maturation in females injected with

ganglionic extracts differed significantly from their respective controls ($P < 0.05$) (Table 7 and 8).

3) Steroid hormone (17α- hydroxyprogesterone) treatment

Shrimps administered with 17α-hydroxyprogesterone at the rate of 0.1μg/ g body weight were found to exhibit a slight increase in the GSI (0.5324) at the termination of the experiment. The ovaries of control shrimps (ethanol injected) were small, white and thin and their average GSI was 0.336. Histologically, majority of the oocytes in the treated females were in the primary vitellogenic phase (79%), with ova diameter ranging from 40 to 100μ, while some (21%) were in the early vitellogenic phase of maturation (ova diameter 60 to 120μ) (Plate 17). The ovaries in controls contained only oogonia and primary oocytes. Electrophoretic analyses of haemolymph samples from the treated females showed weak expression of FSP after a period of ten days.

Statistical analysis by means of one-way analysis of variance (ANOVA) of the GSI of females treated with 17α-hydroxyprogesterone showed that the ovarian maturation in the treated females was not significantly higher than that of controls (Table 9).

4) Neurotransmitter (5-hydroxytryptamine) treatment

In the present investigation the ovarian index of females treated with 5-HT exhibited significant increase in their ovarian indices compared to the controls. The average GSI of 5HT treated females were 0.7796, which was higher than the control (0.336). The ovary of the females treated with 5-HT produced significant changes in their histology compared to their controls. Histologically the treated females were in the advanced second stage of maturation with nearly all the oocytes entering the early vitellogenic phase of vitellogenesis. These vitellogenic

PLATE 17. Ovaries of females injected with 17-α hydroxy progesterone

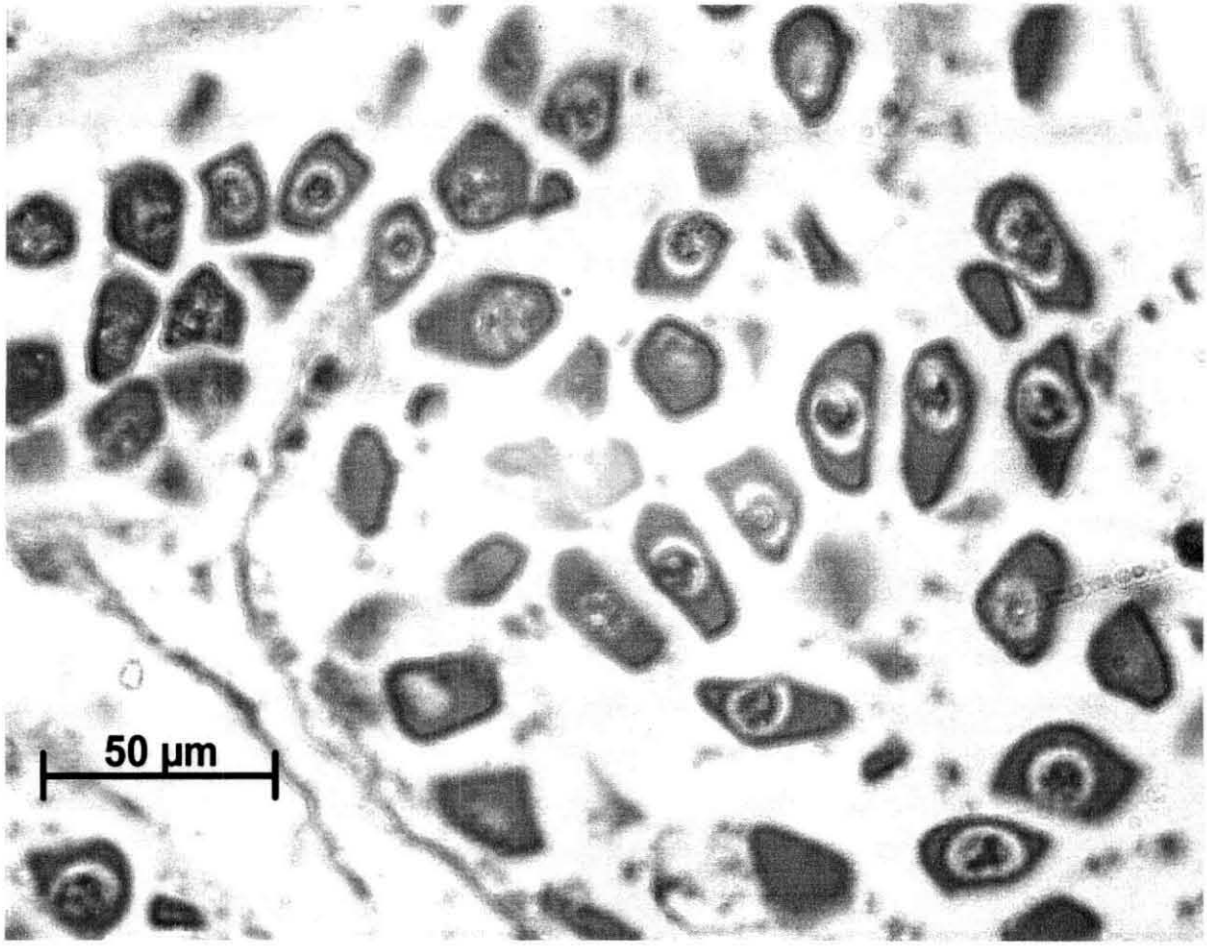
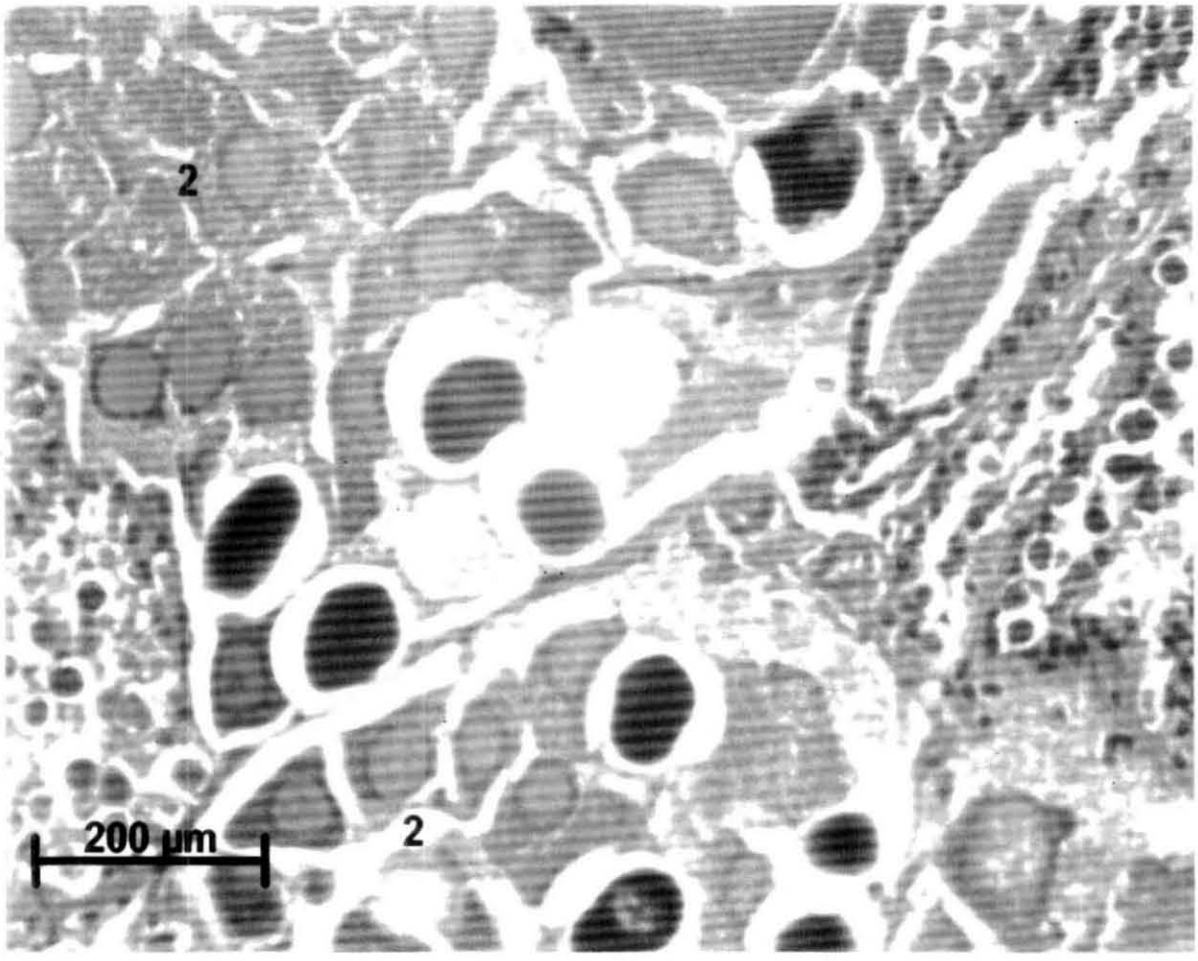

50 µm

PLATE 18a. Ovaries of females injected with cerebral ganglionic extracts (2 – Early vitellgenic oocytes)

PLATE 18b. Ovaries of females injected with 5-hydroxytyptamine


2
200 µm
2

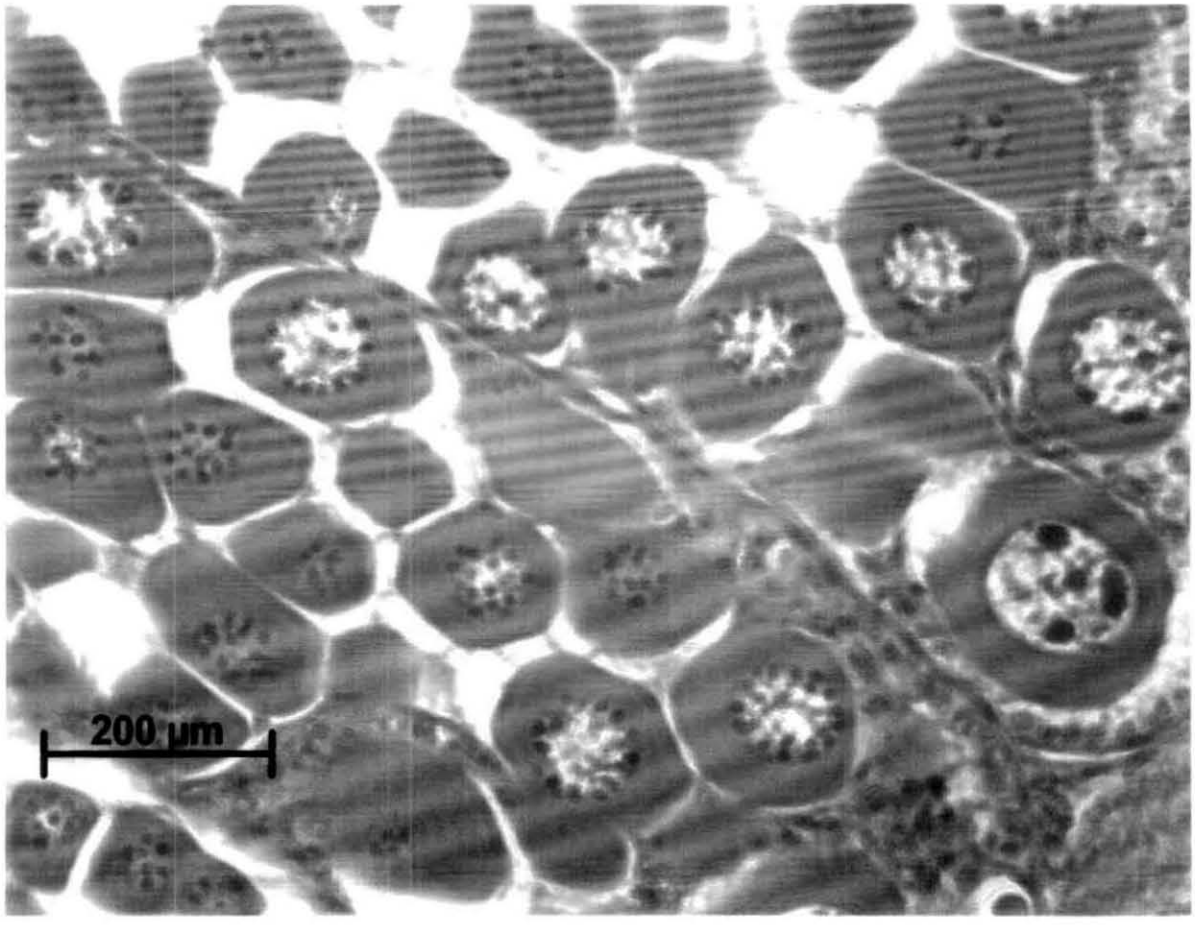

200 µm

Table 7 : Analysis of variance of GSI of females injected with cerebral ganglionic extracts

Source of Variation	SS	df	MS	F	P-value
Between Groups	0.499	1	0.499	9.104	0.024
Within Groups	0.329	6	0.0548		
Total	0.828	7			

Table 8 : Analysis of variance of GSI of females injected with thoracic ganglionic extracts

Source of Variation	SS	df	MS	F	P-value
Between Groups	0.056	1	0.056	6.249	0.047
Within Groups	0.053	6	0.009		
Total	0.109	7			

Table 9: Analysis of variance of GSI of females injected with 17 alpha hydroxyprogesterone

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	0.043	1	0.043	1.811	0.227
Within Groups	0.144	6	0.024		
Total	0.187	7			

Table10: Analysis of variance of GSI of females injected with 5-hydroxytryptamine

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	0.449	1	0.449	17.463	0.006
Within Groups	0.154	6	0.026		
Total	0.603	7			

oocytes were loaded with dense yolk globules and cell size increased significantly (140-188μ), when compared with ovaries of control females. The nuclei of these oocytes were compact with peripherally located nucleoli. Follicular cells were observed surrounding the oocytes. In the control females, the oocytes showed clear nucleus occupying a major portion of the oocyte (Plate 18b).

Electrophoretic analysis of the haemolymph of the 5-HT treated females showed the intense expression of FSP compared to all other treatments in the experiments conducted for ovarian maturation in *P. merguiensis* during the present study (Plate 19).

Analysis of variance of GSI of females treated with 5-HT showed that the ovarian maturation of the treated females was significantly higher than that of controls confirming the stimulatory role of 5-HT on ovarian maturation (Table 10).

DISCUSSION

1) Eyestalk ablation treatment

In the present study eyestalk ablation in *P. merguiensis* females collected from brackishwater ponds enhanced gonadal maturation and spawning. This was presumably due to the decrease in the levels of hormones, which inhibited moulting and gonadal development (Adiyodi, 1985). Similar experiments conducted in *P. monodon* (Joseph, 1996) using wild and pond reared broodstock in full strength sea water showed that gonad maturation was enhanced by eyestalk ablation, though the progress was slower in these animals than that observed in wild animals collected from open sea. In contrast to this, the signs of ovarian maturation in pond reared *P. merguiensis* was more or less similar to that of wild caught shrimps.

PLATE 19. Comparative PAGE analysis of vitellogenin expression in the haemolymph of females subjected to different induced maturation techniques

Lane 1: Mature female

Lane 2: Control

Lane 3: Females treated with 5-HT

Lane 4: Females injected with cerebral ganglionic extracts

Lane 5: Females injected with 17α-hydroxyprogesterone

Lane 6: : Females injected with thoracic ganglionic extracts

→ Vitellogenin

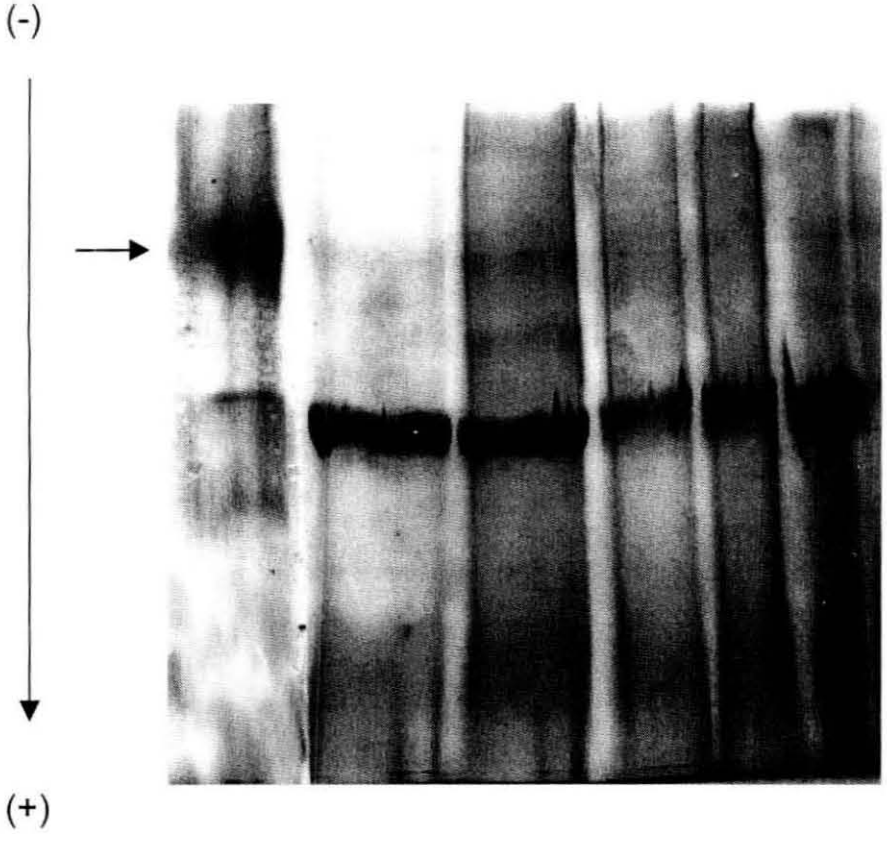


Table 11: Gonad indices and ova diameter of females subjected to different induced maturation experiments

Treatment	GSI	Ova diameter (μ)
Eyestalk ablation		
(I) Warmer months	2.0 - 3.2	110 - 200
(ii) Colder months	1.2 - 2.0	80 - 110
Cerebral ganglionic extract	0.4 - 1.32	75 - 188
Thoracic ganglionic extract	0.4 - 0.69	56 -152
17 alpha hydroxyprogesterone	0.24 - 0.75	40 -120
5- hydroxytryptamine	0.52 - 1.04	140 -188

Visible signs of ovarian maturation were seen in ablated females within three to five days after ablation when the experiment was conducted in bay waters. The time taken for the entire maturation process was 3-5 days, which was comparable to that of wild shrimps in captivity (Lim *et al.*, 1987). It has been noted that the water quality also plays a role in full achievement of the process. The present findings were in agreement with the report of Emmerson (1983) that ablated penaeid shrimps (*P. indicus*) showed signs of ovarian development almost immediately, but it took 1-2.2 months or 2 or 3 moult cycles for unablated females to develop and often never beyond ovarian stage 3. A gap of 10 days to 2.7 months for unablated controls and females caught from the wild probably represents the length of time for eggs to fully mature in a reproductive season (Primavera, 1985). However, reported effects of ablation on fecundity by different penaeids are conflicting (Alikunhi *et al.*, 1975; Lumare, 1979; Emmerson, 1980; Primavera, 1982; Browdy and Samocha, 1985a,b; Browdy *et al.*, 1986).

During the present investigation, the shortest period observed from ablation to the onset of maturation was 3 days and first spawning occurred within 5 days in pond reared animals. In *P. monodon*,the minimum time taken for the onset of spawning was 25 days in wild caught shrimps whereas animals collected from filtration pond took 30 days to reach late vitellogenic stage of ovarian development. It was reported that all of them used out-door ponds or cages, presumably with high light intensity to mature ablated *P. monodon*. This is presumably why they took longer periods than that reported by Primavera (1978). Muthu and Laxminarayanan (1982) reported that *P. monodon* collected from filtration pond took 66 days to mature. They concluded that frequent power failures leading to disruption of the recirculatory system collapsed the water quality, which delayed the maturation process. Lumare *et al.* (1993, 1996) studied the feasibility of stimulating ovarian maturation and reproduction in *P. monodon* by eyestalk ablation in " Valle". Similarly, the results of the present experiments demonstrated the feasibility of ovarian maturation and courtship stimulation in a high percentage

of eyestalk ablated population of *P. merguiensis* in captivity, with the production of larvae, by a relatively simple method.

Histological studies conducted provide evidence for the impact of unilateral eyestalk ablation in ovarian maturation. However, it did not cause uneven development or atresia of the ovary after ablation under the experimental conditions during the warmer months. The presence of large (110-200μ) oocytes in the ovaries of ablated shrimps after spawning, and their absence in the unablated group, indicated that ablated groups released fewer eggs per spawn.

The reproductive cycle of a species is a genetically controlled response to the environment. Environmental factors are known to govern the gametogenic cycle of marine crustaceans. Light intensity and spectrum have been proved to be effective on the reproduction and interdependent on the suspended and dissolved organic material (Bray and Lawrence 1992). Stimulation to reproduction in *P. indicus* by decreasing the light intensity has been reported by Emmerson (1983). Pudadera and Primavera (1981) reported induced ovarian maturation (stage III) in unablated females with blue and natural light than with red light, indicating light of smaller wavelength to have a stimulatory effect on gonadal maturation. Similarly in the present study, eutrophy conditions caused by phytoplankton bloom and high chlorophyll concentration in the bay may have allowed the light of longer wavelengths to scatter and thereby allowing light of the smaller wave lengths to pass through and probably enhance maturation in the shrimps.

In terms of the size of animals, the rate of achievement of gonadal maturation in large animals was much greater compared to the smaller shrimps during the present investigation. The minimum size for maturation in *P. merguiensis* was reported to be 23mm carapace length (CL) (Tuma, 1967). Results of the second experiment where females below the reported size for

maturation were subjected to eyestalk ablation, no response in ovarian maturation was observed, though the animals were maintained in the same conditions in which larger females subjected to ablation were maintained. Thus, larger females (120-150mm in total length and 23-35mm carapace length) were found to attain full maturity and spawn within a period of 3-8 days while no signs of maturation was seen in the smaller groups. These results indicated the need of older and larger females for successful production of postlarvae from pond reared brood stock. In the present investigation animals of more than 23mm CL size mature faster than smaller animals.

The females ablated in the colder months showed a delay in response to eyestalk ablation technique. The ablated females responded only after a period of 7 days. Studies indicate that a reproductive response is produced through a relative interaction of environmental factors especially temperature, light, salinity and food and endogenous factors of an organism (Emmerson 1980, 1983). Thus, the pattern of reproductive cycle in a species is apparently determined through the co-ordination of the successive reproductive events with changes in the external environment. The influence of some environmental factors on the growth of gonad has been determined experimentally. Temperature and light are considered to be the most important physical factors influencing gonad maturation of marine organisms and the biological effects of these factors are complex and wide ranging. Nevertheless, the mechanisms of co-ordinating the physiological process underlying the events within the organism and the changes in the environment are not clearly understood.

In land based confined systems, environmental and nutritional parameters of shrimps are often difficult to maintain. The circulation of water in the experimental unit during the tidal fluxes flushed away the accumulated waste in addition to bringing in nutrient and oxygen rich water, thereby reducing stress and benefiting the reproductive performances. The results obtained in the present study suggested that a system incorporating unilateral eye stalk ablation, high salinity,

good water quality, reduced light intensity and optimum temperature are sufficient to induce maturation in females above the length of first maturity of *P. merguiensis* in captivity. Regarding the effect of broodstock source on maturation, in this study, it was found that pond reared and wild caught brood stock performed comparably well provided the size and age were adequate. These findings were in accordance with the similar observations made by Menasveta *et al.* (1994) in *P. monodon*, Rangnekar and Deshmukh (1968) in *Scylla serrata* and Bomirski and Klek (1974) in *Crangon crangon*.

2) Ganglionic extract treatment

Advanced ovarian development and a high rate of GSI were observed in female *P. merguiensis* injected with cerebral, thoracic ganglionic extracts within the experimental period. The water temperature, salinity and dissolved oxygen were same for the treated and control shrimps in the study period and hence the response of the shrimps to the treatment were not due to environmental conditions.

The GSI observed in females receiving cerebral (0.9352) and thoracic (0.525) ganglionic extracts after 10 days were high compared to the control (0.357). This indicates the possibility that ovarian maturation can be induced and accelerated by injecting ganglionic extracts (cerebral and thoracic ganglion) prepared from vitellogenic females. The histological observations observed in the cerebral treated groups were similar to those observed in females undergoing maturation, thereby suggesting normal development or vitellogenesis occurring in these shrimps under the stimulation of the ganglionic extracts injection. But in the females receiving thoracic ganglionic extracts, majority of the oocytes were still in the previtellogenic stage with less progress in maturation after a period of ten days.

These results suggested that the brain together with thoracic ganglion fabricate the ovary stimulatory principle.

The stimulatory factor present in the cerebral and thoracic ganglion extracts believed to be responsible for the ovarian maturation are the hormones namely gonad stimulatory hormone (GSH) in the thoracic ganglion and Gonad-stimulating hormone-releasing hormone (GSH-RH) in the brain. GSH acts directly on gonad in crustaceans while GSH-RH acts on ovary indirectly by regulating the release of GSH. These hormones are reported not to be species specific in activity in crustaceans (Yano, 1988; Yano and Wyban, 1992). The results observed in the present experiment were thus comparable with the earlier reports of precocious vitellogenesis stimulated by implants of the thoracic and cerebral ganglion *in vivo* in several species like in the crabs *Potamon dehaani* (Otsu, 1960), *Paratelphusa hydrodromous* (Gomez, 1965), *Libinia emarginata* (Hinsch and Bennett, 1979); *Uca pugilator* (Eastman-Reks and Fingerman, 1984) and in shrimps *Parapenaeopsis hardwickii* (Nagabhushanam and Kulkarni, 1982), *Penaeus vannamei* (Yano, 1993), *P. japonicus* (Yano, 1992) and *Metapenaeus affinis* (Nagabhushanam *et al.*, 1992). The effect of these ganglionswere reported to be related to the stage of reproductive activity of the donor female as cerebral and thoracic ganglion extracts from immature females failed to stimulate ovarian growth in the treated groups, thus suggesting the secretion of the stimulatory factor only in vitellogenic females.

In *P. merguiensis*, full maturation and spawning was not achieved by injection of brain and thoracic ganglionic extracts into the females, even though indications of ovarian maturation initiation were observed. This clearly confirms the gonad stimulatory role of these ganglions on ovarian maturation. The delay in achieving full maturation may be due to the low dose of a single injection administered to the shrimp. Repeated injections or implantation were usually done in earlier reports, but in the present experiment a single injection was resorted to reduce stress.

A period of 10 days post-initial treatment was chosen in the present study for sampling as normally the effects of eyestalk ablation on ovarian maturation was detected within 5-8 days. This period was considered sufficient to detect the effects due to ganglion extracts, if any, on ovarian maturation. The delay in observing significant signs of ovarian maturation during the experimental period probably indicated that the experimental time (10 days) was too short for exhibiting full maturation in the treated shrimps or the ovarian maturation factor (OMF) reported to be present in these ganglions may have been metabolised or degraded after the initial triggering of ovarian maturation by the recipients animals. Another reason for the delay in maturation was probably due to the use of crude extracts of ganglion. Nevertheless, the present finding suggested that ovarian stimulatory principles were harboured in the brain and thoracic ganglion of *P. merguiensis* of vitellogenic females.

3) Steroid hormone (17α-Hydroxy progesterone) treatment

Vertebrate hormone has been reported to have a vitellogenesis stimulating effect in various crustaceans, but their mode of action is still unknown. Results of the present study revealed an increase in the GSI in shrimps treated with 17α-hydroxyprogesterone suggesting a stimulatory effect on the oogenesis by the hormone. Other workers have also mentioned similar observations on the stimulation of oogenesis by 17α-hydroxyprogesterone. Yano and Chinzei (1987) have reported a significant increase in vitellogenin concentration in the sera in eyestalk ablated Kuruma shrimp *P. japonicus* injected with hydroxyprogesterone compared to the unablated ones. A similar observation was also made on vitellogenesis in greasyback shrimp *Metapenaeus ensis* (Yano, 1985), *Parapenaeopsis hardwickii* (Kulkarni *et al.*, 1981) and *P. merguiensis* (Chan and Lim, 1988).

17α-hydroxyprogesterone, for which progesterone acts as a precursor, also stimulates vitellogenesis. Conversion of progesterone into 17α-hydroxyprogesterone has been demonstrated in the ovaries of spiny lobster *Panulirus japonicus* (Kanazawa and Teshima, 1971) and in crab *Portunus trituberculatus* (Teshima and Kanazawa, 1971). It is however, unclear whether progesterone acts directly or as a precursor to stimulate vitellogenin synthesis and/or release into haemolymph. Junera *et al.* (1977) deduced the existence of vitellogenin stimulating ovarian hormone, which controlled vitellogenin synthesis in the amphipod *Orchestia gammarella.* Based on the present observations, it was deduced that 17α-hydroxy progesterone stimulated vitellogenin synthesis and its release into the serum resulting in the increase in the GSI observed in the treated females in comparison to the controls. The mode of action of these hormones in penaeid shrimps including *P. merguiensis* can be suggested to be similar to that in vertebrates where they activate the brain, which in turn stimulates the ovary by releasing gonadotropins.

Again in the present study, the increase in the GSI can be related to the stimulatory effect of 17α-hydroxyprogesterone, but full maturation and spawning was not obtained in *P. merguiensis*. The failure to detect FSP during electrophoretic analysis also indicated the poor response of the shrimps to the steroid treatment. It also indicates that optimal doses of 17α- hydroxyprogesterone to be injected and injection sequence to induce ovarian maturation and spawning may vary among species.

4) Neurotransmitter (5-hydroxytryptamine) treatment

Administration of neurotransmitter 5-hydroxytryptamine to female *P. merguiensis* showed pronounced ovarian development than did the concurrent controls injected with saline. The average GSI of the treated females was 0.7796. A significant increase in ovarian index and oocyte size of crayfish *P. clarkii* given

5-HT was reported by Kulkarni *et al.* (1992). Richardson *et al.* (1991) have reported an increased dose dependent ovarian development in the fiddler crab *Uca pugilator,* while Kulkarni *et al.* (1992) and Kulkarni and Fingerman (1992) have also given supporting evidence showing the stimulatory role of 5-HT in the reproduction of female crustaceans. Ragunathan and Arivazhagan (1999) reported the effect of eyestalk ablation together with 5- hydroxytryptamine on freshwater crab *Paratelphusa hydrodromous*. It is suggested that 5-HT, which is present in the central nervous system of this crayfish exerted its effect indirectly, by stimulating release of the ovary-stimulating hormone. Similarly in the present study the significant increase in the GSI after 15 days of administration of 5-HT may also be concluded to be due to its stimulatory effect on central nervous system, resulting in induction of ovarian maturation in *P. merguiensis*. Based on the experiment a serotonin injection programme seems to be a practical alternative to eyestalk ablation.

From the present investigation, it is evident that endocrine manipulations play an important role in the gonadal development, by acting either directly or indirectly on the target organs. The response of *P. merguiensis* to these treatments during the present study indicated the possibility of closing the life cycle of *P. merguiensis* in captivity, which has greatly improved the outlook for successful commercialisation of the culture of this species. The advances made in controlling reproduction in *P.* merguiensis in the present study reaffirms its selection as a species for culture in captivity. In spite of the above accomplishment of induced ovarian maturation of *P. merguiensis* in captivity it is strongly felt that much more remains to be done to have a thorough control of the reproductive performance of the species.

Chapter 4

LARVAL REARING OF *PENAEUS MERGUIENSIS*

LARVAL REARING OF *PENAEUS MERGUIENSIS*

Biology of aquatic organisms in an ecosystem is profoundly influenced by several abiotic factors such as salinity, temperature, pH, etc. and their distribution is determined by the degree of influence exerted by these factors either independently or in combination (Gunter, 1950; Venkataramaiah *et al.*, 1972; Dall *et al.*, 1990; O' Brien, 1994; Tsuzuki and Cavalli, 2000). Larval rearing of an organism is the most difficult and important stage in an economic farming operation. Development of improved hatchery techniques has helped to produce shrimp seed in sufficient numbers to supply farmers, but still a wide gap between stocking and harvest remains to be bridged to obtain the potential yield. Maximising the growth rate and minimising the mortality of postlarvae are the twin factors to achieve maximum yield.

A review of literature on the biology of penaeid shrimps reveals that over 70% of the penaeid shrimps are euryhaline encountering a wide variety of salinities throughout their life cycle. Their semi-catadromous life involves both high and low saline ecosystems as most penaeid shrimps rely on stability of marine environment for spawning and early larval developments. Subsequently, the larvae pass through several planktonic stages and move towards the coast (Garcia and Le Reste, 1981) where they usually arrive as postlarvae. Postlarvae then migrate to lower saline estuarine nursery areas by means of tidal currents where they are recruited into the submerged vegetation that is used as a zone of growth (Mc Tigue and Zimmerman, 1991).

Variations in salinity and temperature are the key factors that play a vital role on the survival, growth and distribution in aquatic animals (Kinne, 1970a, b; Charmantier, 1998). The distance that maturing stocks move offshore away from the estuarine areas varies among species (Kutkuhn, 1966) and hence the extent to which the planktonic larvae encounter differences in temperature and salinity also

varies. In species, which spawn well offshore, the entire larval development is completed in marine waters at levels of salinity and temperature that do not vary greatly. In contrast to this, the larvae of species which spawn in or close to estuarine area, may encounter rapid changes in temperature and salinity. The ability of each developmental stage to adapt to environmental conditions and their fluctuation is thus a major adaptive process, which determine the successful establishment of a species in a given habitat. In spite of the economic importance of penaeid shrimps to fisheries, studies related to their larval ecology have been largely confined to descriptions of planktonic stages (Anderson *et al.*, 1949; Nandakumar *et al.*, 1989; Chong, 1991).

Like several other penaeid shrimps, *Penaeus merguiensis* spawns offshore and uses estuarine nursery areas for postlarval and juvenile growth (Figure 3). The species is also characterised by large fluctuations in the population size caused by mortalities induced by a variety of biotic and abiotic factors at each stage of the life history. Information available on *P. merguiensis* is mainly from the waters of Australia and Papua New Guinea (Tuma, 1967; Munro, 1975; Staples, 1980). Moreover, the larval studies on Banana shrimp *P. merguiensis* from India are scanty (Raje and Ranade, 1972; Vinod *et al.*, 1996; Sadhana and Neelakantan, 1996, 1997). To understand and optimise production conditions, it is necessary to investigate the effects of salinity and temperature on growth, survival and overall production of shrimps. Though certain information on salinity requirement of this species is available, studies on early larval and post larval stages are inadequate. Hence, the present study was conducted with the following objectives

1) To study the effect of salinity and temperature on the hatching success, survival and rate of development of the early developmental stages of *P. merguiensis* and
2) To study the effect of salinity on the growth and survival of postlarvae of *P. merguiensis*.

1) EFFECT OF SALINITY AND TEMPERATURE ON EARLY DEVELOPMENTAL STAGES (NAUPLIUS TO POSTLARVAE) OF *P. MERGUIENSIS*

Early stages of development are the most sensitive phase in the complex life cycle of marine invertebrates. Hudinaga (1942) first achieved rearing the larvae of penaeid shrimps in laboratory condition in *P. japonicus*. The studies of larval development of penaeid shrimps have in recent years attained much significance with their increasing importance in aquaculture. Larval stages of *P. merguiensis* were described by Raje and Ranade (1972), Motoh and Buri (1979) and Ruangpanit *et al.* (1984) while Lui and Lui (1994) compared the larval development of *P. merguiensis* with those of *P. chinensis* and *P. penicilatus*.

The successful establishment of a species in a given habitat depends on the ability of each of its developing stages to adapt to the existing environment (Charmantier, 1998). High mortality rates during the early larval stages of penaeid shrimps have been encountered in hatcheries. In order to maximise survival of the developing stages, larvae should be reared close to optimal conditions. Different strains of the same species may have difference in the requirement of optimal conditions for growth. There are numerous studies on the effect of salinity alone or in conjunction with other abiotic factors on the growth and survival of non-penaeid crustaceans (Lester and Pante, 1991) and a few studies on commercially important penaeid shrimps such as *Penaeus setiferus* (Zein- Eldrin and Griffith, 1968), *P. aztecus* (Venkataramaiah *et al.*, 1972), *P. vannamei* (Oogle *et al.*, 1988; Mair, 1980), *P. stylirostris* (Mair, 1980), *P. indicus* (Kumulu and Jones, 1995), *P. merguiensis, P. esculentus and Metapenaeus bennettae* (Dall, 1981), *P. monodon* (Cawthrone *et al.*, 1983), *P. japonicus* and *P. chinensis* (Charmantier- Daures *et al.*, 1988) and in *P. semisulcatus* (Harpaz and Karplus, 1991). The optimal environmental condition for growth, thus,

appears to be species-specific and differ between life-history stage and season (Costlow *et al.*, 1960; Bas and Spirak, 2000). Laboratory and field studies of responses of eggs and larvae of marine organisms to the combined effects of temperature and salinity would lead to a greater understanding of the significance of these factors in determining survival during early larval development. Thus, defining these optimal conditions for culture of euryhaline marine species may be fundamental for developing the rearing protocol for these species.

Salinity has been reported to have a dramatic effect on the percentage of eggs that hatch after spawning and the survival of the resulting larvae (Preston, 1985). Choo (1987) reported the development of normal eggs and healthy nauplii when spawning and egg incubation were carried out in water of salinity greater than 20‰. But at a salinity level of 18‰ only few eggs were produced, which were weak and died subsequently. Pillai (1991) reported eggs of *Macrobrachium idella* to hatch out in freshwater as well as in water with a salinity range of 5- 35‰. Eggs and larvae of *P. japonicus* were reported to be less tolerant to changes in salinity than changes in temperature (Hudinaga, 1942). The response of different larval stages of a species to changes in abiotic factors varies. During the nauplius stage, the rate of development is mainly influenced by abiotic factors such as temperature and salinity as the larvae do not feed at this stage. On reaching the protozoeal stage feeding commences and the rate of development is also influenced by the abundance of suitable food in addition to salinity and temperature.

Salinity tolerances become broader as larval development proceeds. Protozoea had the lowest tolerance to changes in salinity, while mysis stages were the least affected (Gopalakrishnan, 1976; Preston, 1985). Salinity below 28‰ and above 48‰ have been reported to be lethal for protozoea of *P. monodon* while mysis larvae were reported to withstand

20‰ (Parado-Estepa *et al.*, 1993). Preston (1985) described a shift in peak survival from around 35‰ in naupliar stages to 30‰ or less for mysis stage in *Metapenaeus bennettae*. Protozoea of *P. semisulcatus* was reported to tolerate hypersaline conditions for a few days, but in general larval growth and development was better in salinities between 25 and 40‰ (Kumulu *et al.*, 1999). Similar studies were conducted on the combined effects of temperature and salinity on survival, growth and development on the early developmental stages of several crustacean species such as *Rhithropanopeus harrisii* (Goncalves *et al.*, 1995), *Sesarma reticulatum* (Paula *et al.*, 1992), *Macrobrachium olfersii* (Montenegro and Moreira, 1992), deepwater shrimp *Pandalus borealis* (Rasmussen and Tende, 1995) and lobster *Homarus americanus* (Mackenzie, 1985).

In general, temperatures ranging from 24^{0}C to 32^{0}C and salinities ranging from 27 to 34‰ have been found suitable for development of early stages of penaeid larvae (Hudinaga, 1942; Cook and Murphy, 1969). Survival of *P. aztecus* was best at 28 to 30‰ (Cook and Lindner, 1970). Kumulu and Jones (1995) reported a salinity of 25‰ as optimal for larval culture of *P. indicus* (PZ1- PL1). Beard *et al.* (1977) also reported similar result that gradual reduction in salinity to 25‰ by the time the postlarval stage was reached, to be beneficial. It is thus evident that proper attention should be paid to these physical properties of seawater used for rearing the larvae for large-scale aquaculture practices. Hence, the present study was conducted to examine variation in survival and development of early larval stages of *P. merguiensis* in response to the combined effects of temperature and salinity under laboratory conditions.

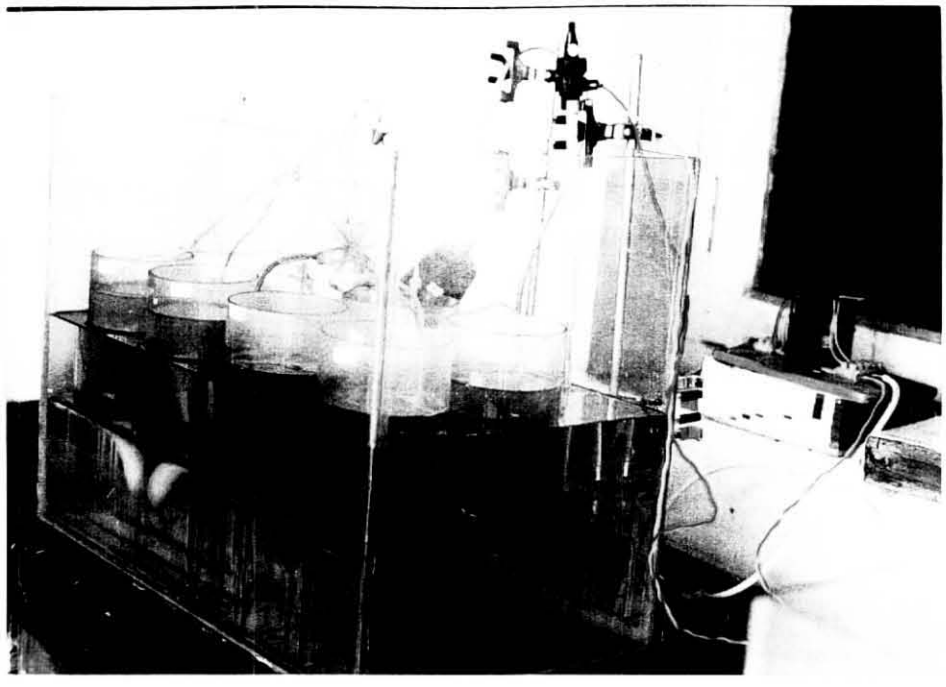
MATERIALS AND METHODS

Gravid females of *P. merguiensis* were captured from commercial fishing grounds off Karwar (Latitude $14^0$48'N and Longitude 74^0 6' E) and brought to the laboratory and allowed to spawn in 100 litre tanks containing seawater of 32- 33‰ salinity and temperature of 29 ± 1^0C. Once eggs were observed in the water column, they were removed by siphoning and passing through a sieve. Three 5ml aliquots were taken to determine egg density. Eggs and nauplii obtained from single spawn in captivity were used for the experiment. The combined effect of salinity and temperature on survival, growth and development were determined in the laboratory using a balanced 4 x 2 factorial design. Two temperature ranges *viz.*, room temperature (29 ± 1^0C) and an elevated temperature (33 ± 0.5^0C) and four salinities (25, 30, 35 and 40‰) were selected to span the environmental range encountered during the spawning season. To avoid abrupt changes in temperature and salinity, larvae were initially placed in 500ml of water at ambient condition. The required temperature and salinity were then adjusted over a period of two hours. Vertical glass troughs of two-litre capacity were used for the experiment (Plate 20a, b). Stocking density in the experiment were 100 eggs/ litre, 100 nauplius/ litre and 50 protozoae/ litre. The stocking densities were below the growth limiting density for penaeid larvae (Emmerson and Andrew, 1981).

The required salinity was obtained either by diluting seawater with fresh water or by mixing filter seawater with sea salt. Salinities were adjusted daily as necessary to keep variation within ± 1‰ and changes were effected by replacing portion of water in the container with equal volume of either freshwater or concentrated seawater. The beakers were maintained at the required temperature adjusted to within ± 0.5^0C using thermostatically

PLATE 20a. Experimental set up to study the effect of different salinity on early larval development of *Penaeus merguiensis* at elevated temperature

PLATE 20b. Experimental set up to study the effect of different salinity on early larval development of *Penaeus merguiensis* at room temperature



controlled water baths. The larvae were fed with the diatom *Chaetoceros* as it has been proved an ideal feed for penaeid larvae with the necessary n-3 polyunsaturated fatty acids (PUFA) required for larval development (Quinitio and Villegas, 1982; Chu, 1989; Cao *et al.*, 1990; Duerr *et al.*, 1992). The algae were introduced to the beakers in all the test groups as soon as the larvae reached the last (sixth) naupliar stage and were continued till the termination of the experiments.

Every day the flasks were emptied and the larvae in each flask were counted and staged according to Motoh and Buri (1979). Size was not used to evaluate the effect of salinity and temperature. Time required for larvae to reach the successive developmental stages and relative survivals of the larvae were used to infer the suitability of salinity-temperature range. Each experiment was continued till larvae at the lowest temperature had either reached the relevant stage or were dead. Larvae were considered dead when limb movement had ceased. Each tank was continuously aerated and maintained at normal day - night illumination. Salinity and temperature were measured each morning using a portable refractometer (ERMA) and a mercury thermometer. Mean stage index (MSI) was calculated with the formula:

$$MSI = (S \times P_s)$$

where S represents the larval stage and P_s represents the proportion of larvae still alive at stage S (Lovett and Felder, 1998; Parado-Estepa, 1998). Absolute values of 1 to 7 were assigned consecutively to the larval stages (protozoea1 to postlarvae 1).

Statistical analyses were performed to assess the effect of salinity, temperature and their interaction on hatching percentage, metamorphism and survival of nauplius to protozoea and also from protozoea to postlarval stage.

RESULTS

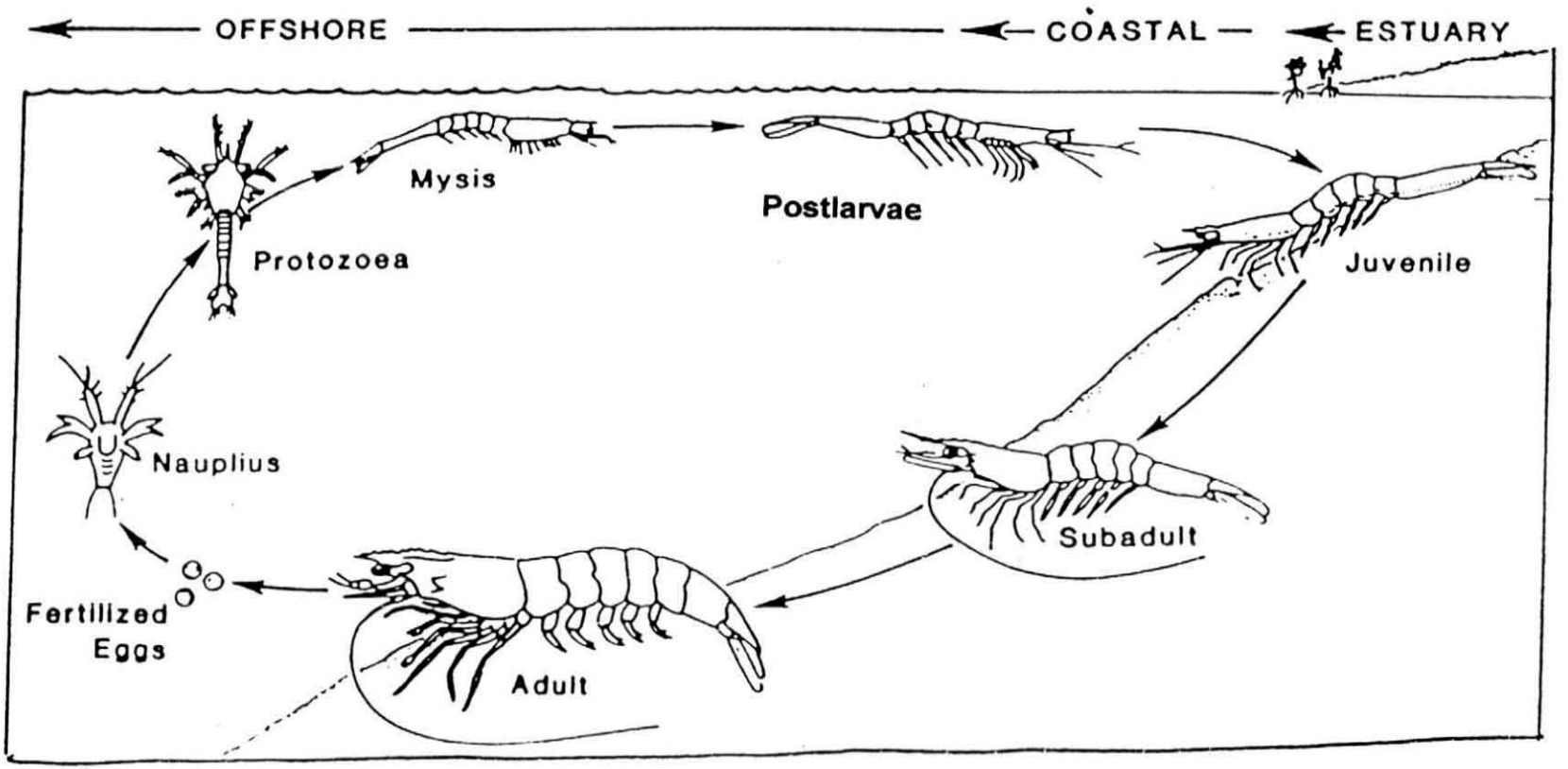
A) Larval stages of *Penaeus merguiensis*

Development for many crustacean larvae consists of gradual morphological changes with successive moults until the first postlarval stage. This process of gradual, but pronounced changes through their larval development can be considered as true metamorphosis (Passano, 1960).

P. mergiuensis followed the general pattern of larval development as in other penaeids with six naupliar stages which subsisted entirely on yolk followed by six feeding stages of three protozoea and three mysis stage before the first postlarvae (Figure 3). As *P. merguiensis* grew from the earliest naupliar stage through protozoea and mysis stages to postlarvae, they developed greater morphological and behavioural resemblance to the adults (Lester, 1984). Raje and Ranade (1972) and Motoh and Buri (1979) provided a detailed description of the larval stages of *P. merguiensis* and hence only the salient features are presented in this thesis (Plate 21).

Egg : The eggs were spherical, white, translucent and demersal with a narrow perivitelline space. They were shed free in water by the mother shrimp. The developing nauplii almost completely filled the cavity within the egg. The embryo developed in about 18- 24 hours depending on the temperature and hatched out as first nauplius (Plate 21a, b).

FIGURE 3. Diagrammatic representation of the life cycle of *Penaeus merguiensis*



Nauplius : The body was pear shaped, unsegmented and dorsally smooth (Plate 21c, d) with three pairs of natatory appendages - the first and second antennae and the mandibles. No spines or processes were present on these appendages for feeding purposes. Mouth was not formed and the larvae depended on internal yolk for development. A pair of caudal setae of equal length was present that extended straight posteriorly.

Six well defined stages of the nauplius were present based on the number of furcal setae. The first nauplius was characterised with non-plumose setae and the antennule bearing two long terminals and one long lateral seta. From the second nauplius onwards the setae became plumose. Details of the number of furcal setae in different naupliar stages were as follows:

Stage	NI	NII	NIII	NIV	NV	NVI
Furcal setae	1+1	1+1	3+3	4+4	6+6	7+7

Protozoea : There were marked developmental changes at the moult of nauplius VI to protozoea I. This stage was characterised by a large carapace followed by a slender thorax and abdomen. The carapace did not cover the thorax completely. Uniramous antennules and biramous antennae with fully segmented exopods were present. Abdomen bifurcated posteriorly, each furca had at le ast seven setae. Three distinct substages of protozoea were identified.

Protozoea I : Eyes were sessile. No frontal organs were apparent. Rostrum and supraorbital spines were absent. Abdomen was unsegmented (Plate 21e).

Protozoea II : Major development at this stage was the appearance of compound stalked eyes, rostrum and orbital spines and segmentation of abdomen. The first five abdominal segments got demarcated while telson was not separated from the last abdominal segment. Uropods were absent (Plate 21f).

Protozea III : A major development at this stage was the separation of telson with biramous uropods as a distinct segment, articulated with the sixth abdominal segment. Spines were present on the abdominal segment. Uropods were also present (Plate 21g).

Mysis : Characteristic features at this stage were the appearance of third maxillipeds, biramous pereopods and the presence of carapace covering the thorax dorsolaterally. The last abdominal segment carried a sharp anal and dorsomedian spine. A long rostrum extending beyond the tip of the eye was also present. Three substages were identified in this stage, the major identification features of which are listed below.

Mysis I : Pleopod buds not developed (Plate 21h).

Mysis II : Unsegmented pleopod buds developed (Plate 21i).

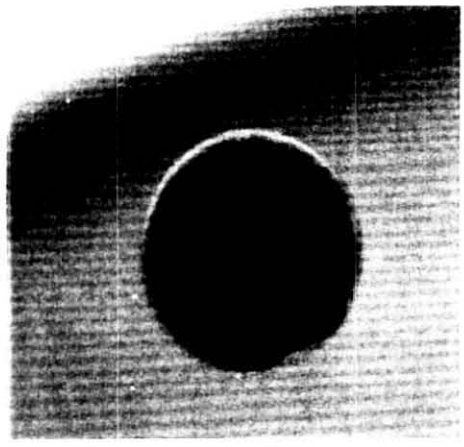
Mysis III : Pleopod buds two segmented, but without any setae (Plate 21j).

B) Combined effect of salinity and temperature on early larval development of *P. merguiensis*

The larvae were exposed to two temperature ranges (33 ± 0.5^{0}C and room temperature, 29 ± 1^{0}C) and four salinity ranges (25, 30, 35 and 40‰). Two way ANOVA was performed to analyse the relative amounts of inter- and intra-variations. The magnitude of F ratio indicated the degree to which the factors in the experimental conditions (temperature and salinity) affected survival and time taken to metamorphose to the next stage.

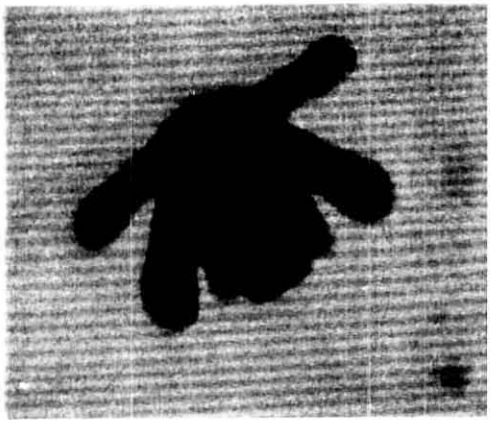
PLATE 21. Larval stages of *Penaeus merguiensis*

a. Egg
b. Gastrula stage
c. Just emerged nauplius
d. Nauplius
e. Protozoea I
f. Protozoea II
g. Protozoea III



a

b



c

d

e

f

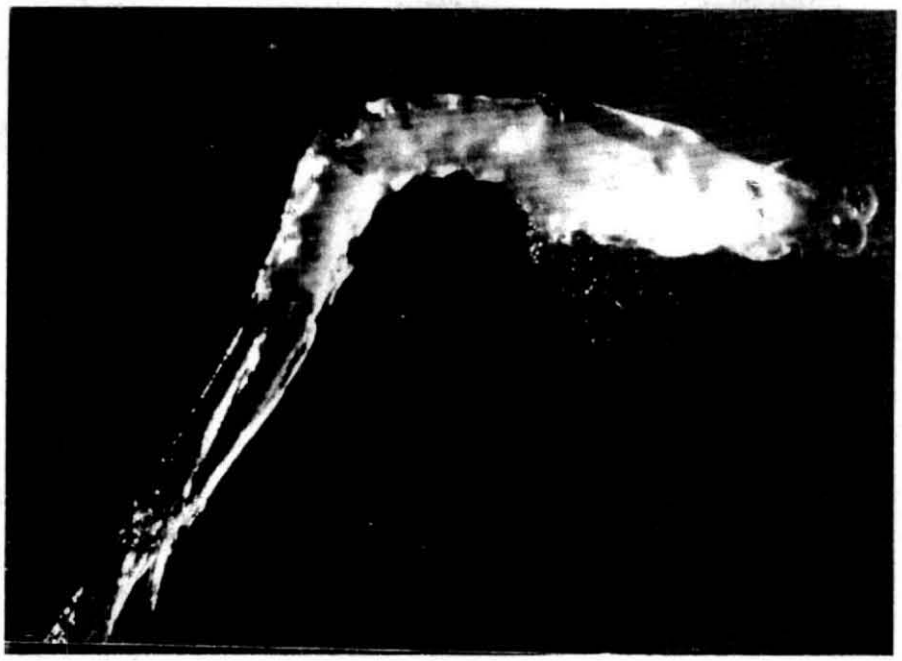
g

Plate 21. Larval stages of *Penaeus merguiensis*

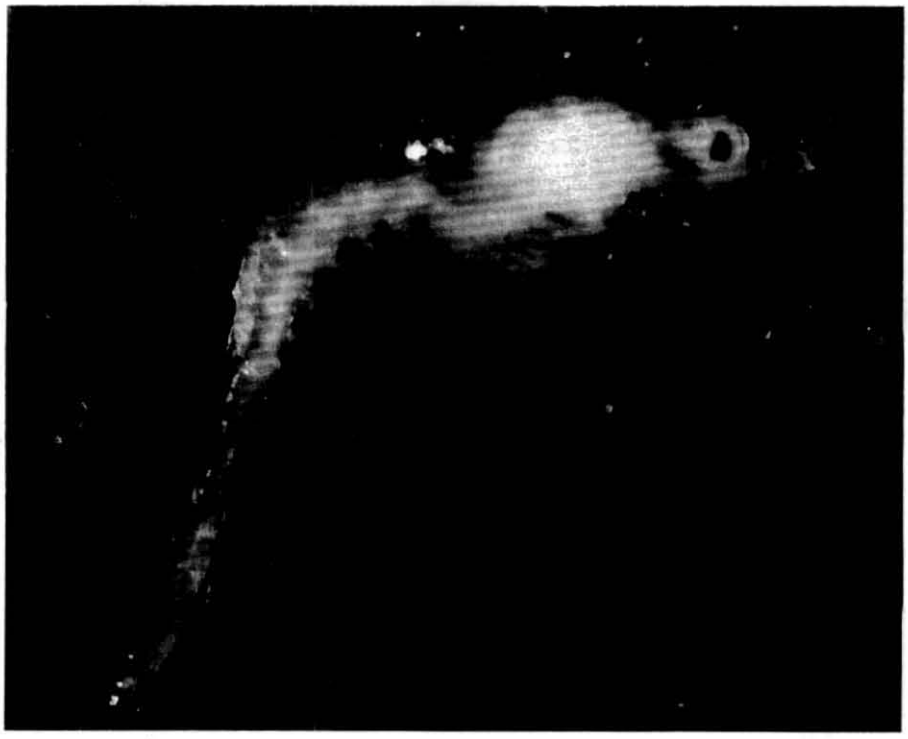
h. Mysis I

i. Mysis II

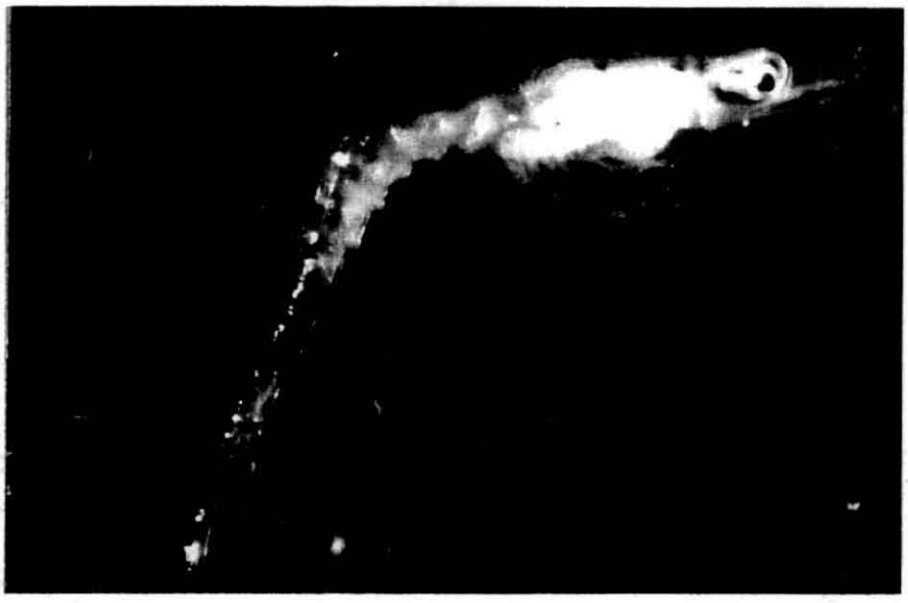
j. Mysis III



h



i



j

(i) Hatching

The hatching success of *P. merguiensis* eggs at different salinity and temperature were studied 24 hours after their introduction to different temperature-salinity combinations. The success rate was with varying percentages at different tested combinations, but followed a general trend of being lowest at the lowest salinity and temperature levels (Figure 4). Maximum hatching success occurred at 35‰ and 33 ± 0.5^{0}C. The percentages of hatch at different salinities tested were in general higher at 33 ± 0.5^{0}C than at room temperature. Survival rates of more than 50% were obtained at 30, 35 and 40‰.

Results of the two-way analysis conducted (Table12) indicated that salinity had a highly significant effect on hatching percentage of the eggs while the effects of temperature and salinity-temperature interactions were not significant at a 5% level.

(ii) Survival of naupliar stages

The survival of naupliar stage acclimatised to different salinity and temperature was studied after a period of 24 hours. Metamorphosis to the next successive stage (protozoeal stage) was successful in all salinities at both tested temperatures, but with varying degree of survival percentages. It followed a general trend of being lowest at the tested extreme salinities. Survival rates of more than 50% were obtained at 30, 35 and 40‰ in both the tested temperatures. In general, percentage of survival in all tested salinities was greater at 33 ± 0.5^{0}C compared to that of room temperature. The highest survival percentage (89%) was obtained at 35‰ at 33 ± 0.5^{0}C followed by 35‰ at room temperature, while the lowest survival was at 25‰ at room temperature. From the results obtained it was evident that the optimum salinity and temperature conditions at hatching (35‰ and 33 ± 0.5^{0}C) also favoured naupliar metamorphism and survival (Figure 5).

Figure 4: Hatching percentage of *Penaeus merguiensis* at different salinity and temperature

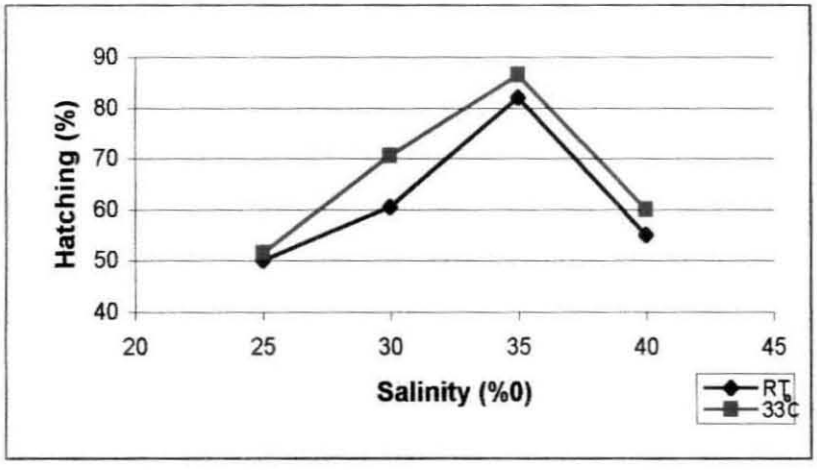
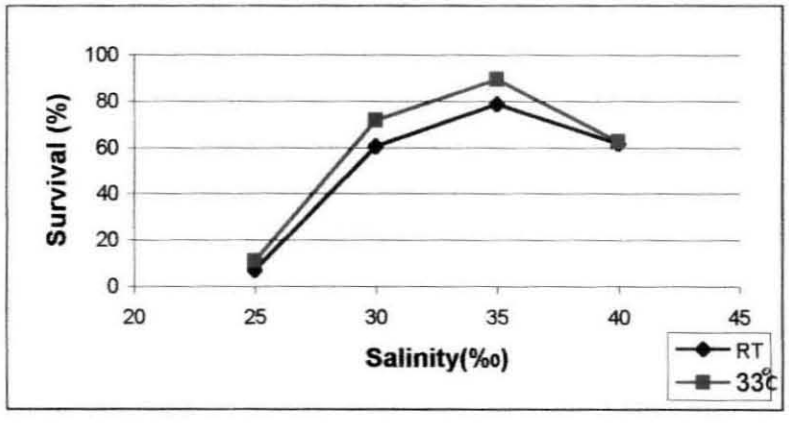


Figure 5 : Survival of naupliar stages of *Penaeus merguiensis* at different salinity and temperature



Statistical analyses of the data by means of two-way ANOVA showed that, of the two factors studied, the effect of salinity on larval survival from nauplius to protozoea I was highly significant. The effect of temperature on naupliar survival was significant at 5% level but salinity-temperature interaction had no effect on the same (Table 13).

(iii) ***Survival of protozoea to postlarval stage***

Both salinity and temperature significantly influenced survival at different stages of metamorphism from protozoea to postlarvae. The larvae metamorphosed to the next successive stage in all the salinities tested, but the time taken to reach postlarvae was significantly greater at room temperature (9-10 days) than at elevated temperature (7-8 days). Larvae maintained at higher salinities, in general, took the minimum time to reach the postlarval stages. The observation was continued till the larvae maintained at the lowest salinity metamorphosed to postlarvae and these were found to increase with decrease in salinity and temperature.

The mean stage index (MSI) of the larvae was determined daily. Larvae subjected to higher temperature had greater MSI values compared to those at room temperature. At the end of the eighth day, the experiment conducted at 33 $\pm$ 0.5^{0}C was terminated. The highest MSI value at elevated temperature was observed at 35‰ followed by 40‰. On termination of the experiments maintained at room temperature on the tenth day, MSI values were more or less the same at 35‰ and 40‰.

Comparison of the progression of MSI over days at different salinity levels at both room temperature and elevated temperature are shown in Figure 6. At 25 and 30‰, the rate of change of MSI over days was higher at room temperature compared to 33 $\pm$ 0.5^{0}C for a period of 4 days after which a higher rate

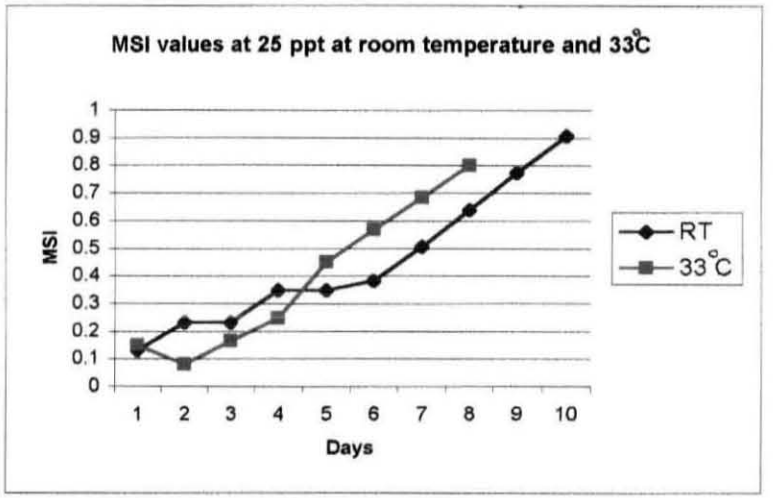
Table 12: Two way anaiysis of hatching percentage of *P. merguiensis* eggs at different salinity and temperature

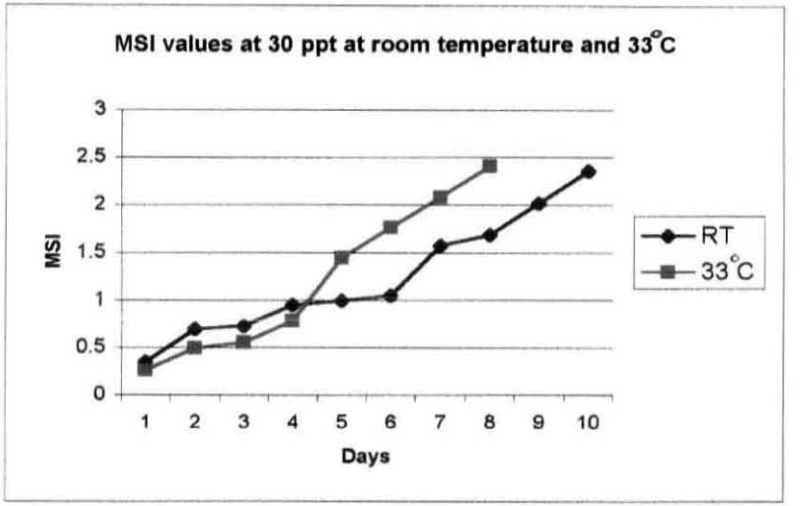
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Salinity	2516.500	3	838.833	23.140	0.000
Temperature	110.250	1	110.250	3.041	0.119
Interaction	37.250	3	12.417	0.343	0.796
Within	290.000	8	36.250		
Total	2954.000	15			

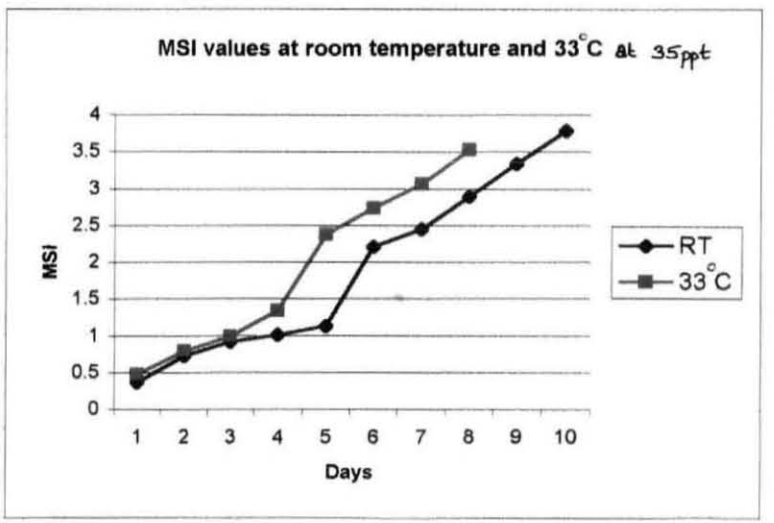
Table 13: Two way analysis of survival rate of naupliar stages of *P. merguiensis* eggs at different salinity and temperature

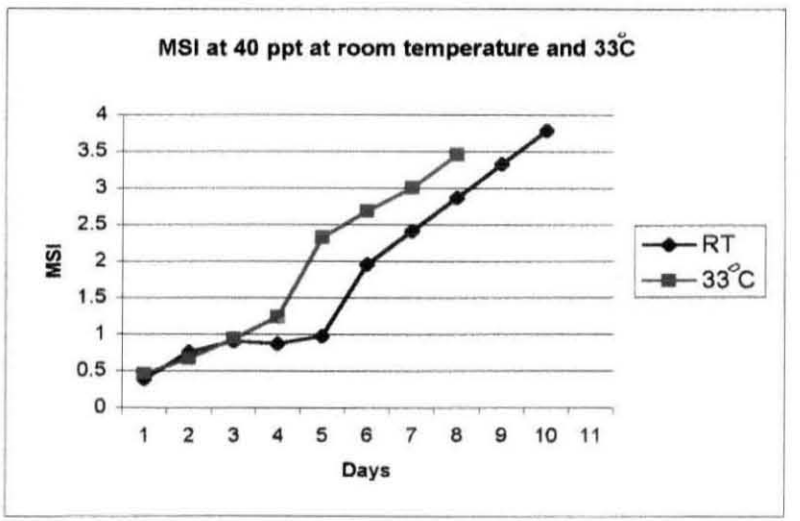
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Salinity	12449.688	3	4149.896	223.563	0.000
Temperature	175.563	1	175.563	9.458	0.015
Interaction	72.688	3	24.229	1.305	0.338
Within	148.500	8	18.563		
Total	12846.438	15			

FIGURE 6: Comparison of Mean stage index of early development stages of *Penaeus merguiensis* at different salinity and temperature









of MSI change over days was obtained at 33 ± 0.5^{0}C. At 35‰, the rate of change of MSI was higher at elevated temperature throughout the experiment while at 40‰, the change in MSI over days was more or less the same at both the tested temperatures for a period of three days, followed by a higher rate at elevated temperature till the termination of the experiment.

Significant differences of the rate of change of MSI over days were tested for the two temperatures by analysis of covariance. From the ANOCOVA, the rate of change of MSI over days at 25‰ (Table 14) was higher at 33 ± 0.5^{0}C (b= 0.10734) compared to that at room temperature (b= 0.08071), but the difference was found to be statistically insignificant at 5% level. At 30‰, the regression coefficient obtained at elevated temperature (b=0.32474) was comparatively greater than that at room temperature (b= 0.20649) (Table 15). F value obtained (F= 15.62) indicated that the difference was significant at 5% level. Similarly at 35‰, the rate of change of MSI over days at elevated temperature (b= 0.46399) was higher than that of room temperature (b= 0.38987) (Table 16) and this difference was also found to be significant at 5% level. At 40‰, testing the equality of regression lines at both room temperature and elevated temperature, revealed a higher regression coefficient at 33 ± 0.5^{0}C (b=0.46384) compared to that obtained at room temperature (b= 0.38765) (Table 17), but this difference was not statistically significant at 5% level.

DISCUSSION

The completion of life cycle of *P. merguiensis* in captivity was related to the species' ability to tolerate variations in environmental conditions. The pattern of larval development of *P. merguiensis* followed closely that of the other species of the genus. Some morphological changes within the same sub-stage were observed in the larval stages similar to that mentioned in *P. monodon*

Table 14. Comparison of regression lines at 25‰. Temperature: room temperature and 33°C. MSI and Days. Test data for the program: EQLINTST for testing regression lines.

Transformation applied : None

ANOVA TABLE

SOURCE	DF	SS-X	SP	SS-Y	b	DF	SS	MS	F
RT	9	82.5000	6.6583	0.5744	0.081	8	0.037	0.00463	
33°C	7	42.0000	4.5083	0.5177	0.107	6	0.034	0.00563	
Total						14	0.071	0.00506	
Pld W	16	124.5000	11.1667	1.0921	0.090	15	0.091	0.00604	
Difference between slopes						1	0.020	0.01974	3.90
Between	1	4.4444	0.2481	0.0139					
W + B	17	128.9444	11.4148	1.1060	0.089	16	0.095	0.00597	
Difference between corrected means						1	0.005	0.00492	0.81

SOURCE	MEAN-X	MEAN-Y	a	b	r
RT	5.500	0.452	0.00778	0.08071	0.967220
33°C	4.500	0.396	-0.08720	0.10734	0.966827
POOLED	5.056	0.427	-0.02069	0.08853	0.955859

Table 15 Comparison of regression lines at 30 ‰. Temperature: Room temperature and 33°C. MSI and Days. Test data for the program: EQLINTST for testing regression lines.

Transformation applied : None

ANOVA TABLE

SOURCE	DF	SS-X	SP	SS-Y	b	DF	SS	MS	F
RT	9	82.5000	17.0354	3.7046	0.206	8	0.187	0.02337	
33C	7	42.0000	13.6392	4.5910	0.325	6	0.162	0.02696	
Total						14	0.349	0.02491	
Pld W	16	124.5000	30.6746	8.2956	0.246	15	0.738	0.04919	
Difference between slopes						1	0.389	0.38918	15.62
Between	1	4.4444	0.0609	0.0008					
W + B	17	128.9444	30.7355	8.2964	0.238	16	0.970	0.06064	
Difference between corrected means						1	0.232	0.23232	4.72

SOURCE	MEAN-X	MEAN-Y	a	b	r
RT	5.500	1.243	0.10739	0.20649	0.974440
33°C	4.500	1.229	-0.23196	0.32474	0.982222
POOLED	5.056	1.237	0.03194	0.23836	0.939709

Table 16 Comparison of regression lines at 35‰. Temperature: Room temperature and 33°C. MSI and Days. Test data for the program: EQLINTST for testing regression lines.

Transformation applied : None

ANOVA TABLE

SOURCE	DF	SS-X	SP	SS-Y	b	DF	SS	MS	F
RT	9	82.5000	32.1646	13.0670	0.390	8	0.527	0.06587	
33°C	7	42.0000	19.4874	9.3136	0.464	6	0.272	0.04528	
Total						14	0.799	0.05704	
Pld W	16	124.5000	51.6520	22.3806	0.415	15	0.951	0.06343	
Difference between slopes						1	0.153	0.15287	2.68
Between	1	4.4444	-0.1907	0.0082					
W + B	17	128.9444	51.4613	22.3888	0.399	16	1.851	0.11567	
Difference between corrected means						1	0.899	0.89929	14.18

SOURCE	MEAN-X	MEAN-Y	a	b	r
RT	5.500	1.878	-0.26639	0.38987	0.979630
33°C	4.500	1.921	-0.16711	0.46399	0.985308
POOLED	5.056	1.897	-0.12067	0.39910	0.957777

Table 17 Comparison of regression lines at 40 ‰ .Temperature: room temperature and 33°C. MSI and Days. Test data for the program: EQLINTST for testing regression lines.

Transformation applied : None

ANOVA TABLE

SOURCE	DF	SS-X	SP	SS-Y	b	DF	SS	MS	F
RT	9	82.5000	31.9812	13.2270	0.388	8	0.829	0.10368	
33°C	7	42.0000	19.4812	9.3586	0.464	6	0.322	0.05374	
Total						14	1.152	0.08228	
Pld W	16	124.5000	51.4625	22.5856	0.413	15	1.313	0.08756	
Difference between slopes						1	0.162	0.16155	1.96
Between	1	4.4444	-0.1032	0.0024					
W + B	17	128.9444	51.3592	22.5880	0.398	16	2.131	0.13321	
Difference between corrected means						1	0.818	0.81793	9.34

SOURCE	MEAN-X	MEAN-Y	a	b	r
rt	5.500	1.835	-0.29750	0.38765	0.968138
33C	4.500	1.858	-0.22946	0.46384	0.982623
POOLED	5.056	1.845	-0.16875	0.39831	0.951652

(Hudinaga, 1942) and in *P. merguiensis* (Motoh and Buri, 1979). These include development of unstalked and compound eyes during the first and second protozoea respectively, development of buds of the third maxilliped and five thoracic somites during the second protozoea, growth of uropods during the third protozoea, development of pleopods during the second mysis and appearance of natatory hairs at the tip of pleopods during the third mysis.

The development and growth of these planktonic larvae took place by moulting as in other crustaceans. The most characteristic feature was the enormous growth taking place during the metamorphosis of nauplius to the first protozoeal stage. During this particular ecdysis, the growth percentage was maximum and the length of the larva almost doubled. Similar observations of maximum growth of the larva during the transformation of the last nauplius into the first protozoea were reported in several penaeid species (Hudinaga, 1942; Raje and Ranade, 1972; Rao, 1973a,b; Mohamed *et al.*, 1978; Muthu *et al.*, 1978a,b).

Roberts (1971) reported each species to have a unique range of salinities suitable for embryonic development and hatching, which bears no *a priori* relationship to the tolerance of adults or larvae. Hatching success and survival of larvae of *P. merguiensis* varied according to temperature and salinity conditions of the rearing medium and was maximum at 35‰, both at room temperature (29 ± 1^0C) and elevated temperature (33 ± 0.5^0C). Other tested salinities retarded hatching success and naupliar survival, though the potency of the effect was different at the two tested temperatures.

Mortality was higher during the development of protozoeal stages than at any other stage of development in *P. merguiensis* as indicated by the low values of MSI. Earlier studies on rearing larvae of penaeid shrimps under laboratory conditions revealed similar results of high mortality rate during protozoeal stage, particularly at the first and third stages (Hudinaga, 1942; Ewald, 1965; Gopalakrishnan, 1976, Omori, 1979, Preston, 1985). In the present study, the

the survival was measured by recording the number of protozoea that survived through to the first mysis stage and it was found that survival through the protozoeal stages in *P. merguiensis* was higher at 35‰ and 33 ± 0.5^{0}C.

The mysis stages showed an increased tolerance to differences in temperature and salinity in comparison with earlier stages as revealed by the high MSI values. The relative indifference of the mysis stages to a range of salinities at both tested temperatures suggested a fundamental change in the mechanisms determining the limits of tolerance of larvae. Similar results of marked increase in salinity tolerance on reaching mysis stage have been reported for many penaeid shrimps, which spawn offshore (Hudinaga, 1942; Ewald, 1965; Gopalakrishnan, 1976; Preston, 1985; Prasad *et al.*, 1988).

From the results obtained, salinity of 35‰ and temperature of 33 ± 0.5^{0}C was found to be optimum for metamorphosis and survival of the early larval stages of *P. merguiensis*. In the present study, at lower salinity (25‰) and temperature (30^{0}C) the larvae metamorphosed into PL1 stage after nine to ten days. At higher temperature, the time taken for the larvae to metamorphose to PL1 stage was reduced considerably to seven to eight days. Thus, salinity and temperature significantly affect the survival of shrimp larvae by determining the length of time the larvae are exposed to planktivores. To achieve high productivity during larval culture of *P. merguiensis* a compromise between optimal rearing conditions for growth and survival must be reached (Staples and Heales, 1991). The study thus, reveals that at least through its early larval stages *P. merguiensis* needs oceanic salinities (35‰) and higher temperatures for optimum growth and survival.

1) EFFECT OF SALINITY ON THE GROWTH AND SURVIVAL OF POSTLARVAL STAGES OF *P. MERGUIENSIS* UNDER LABORATORY CONDITION

The postlarval stage of penaeid shrimp, in which growth is faster, is spent in a relatively unstable environment, the estuary. Inconsistencies in growth and survival of penaeid shrimps in ponds can be attributed to many causes, the most important of which appears to be salinity or a combination of salinity and temperature (Venkataramaiah *et al.*, 1972). In tropical countries like India, among the various abiotic factors salinity assumes greater importance, influencing the postlarval stages as they ascend the brackishwater areas after completion of their larval phase in the sea. Salinity plays a vital role in growth and survival during larval and juvenile phases by affecting their functional and structural responses thereby influencing their survival, growth and distribution (Gunter, 1967; Fry, 1971). Temperature, the predominant abiotic parameter in the tropical environment, has been reported to have no significant role in the occurrence of postlarvae in the estuarine conditions (Bhattacharya and Kewalramani, 1976). Studies of the response of juveniles to different environmental factors can lead to better understanding of stock fluctuations and can be used to develop predictive models. They can also provide information on the optimum environmental factors to maximise the productivity of animals in culture.

Although estuaries are ideal nursery areas for juveniles, they are generally less saline than seawater and improved growth at these intermediate salinities have been reported for many penaeid species (Venkataramaiah *et al.*, 1972; Nair *et al.*, 1975; Gopalakrishnan, 1976; Dall, 1981; Kuttyamma, 1982; Raj and Raj, 1982; Chakraborti *et al.*, 1986a, b; Rajyalakshmi and Chandra, 1987; Harpaz and Karplus, 1991; Staples and Heales, 1991; Bray *et al.*, 1994; Vijayan and Diwan, 1995; Samocha *et al.*, 1998). Abundance of shrimps is reported to be

greater in low salinity (Gunter, 1961) and frequently this relation has been interpreted to mean that low salinities are necessary for young shrimps to survive in estuaries.

Shylaja (1989) reported juveniles of *P. indicus* to tolerate a salinity range of 3.9 - 40.7‰ on sudden exposure to different salinities from a pre-acclimated salinity of 20‰ and a hundred percent survival was reported in a salinity range of 6- 37.7‰, while Lakshmikantham (1982) reported postlarvae of *P. indicus* to tolerate a salinity range of 4- 50‰ under normal conditions. Venkataramaiah *et al.* (1972) reported highest growth rates of *P. aztecus* in lower salinity levels (8.5 - 17.0‰) under normal temperature (26^0C). Nair *et al.* (1975) and Vijayan and Diwan (1995) also reported significant increase in growth in *P. monodon* in low salinity levels (10‰ and 15‰ respectively). Similarly higher growth rate was reported at 25‰ in *P. indicus, P. semisulcatus* and *P. monodon* (Raj and Raj, 1982) . Dall (1981) interpreted the low salinity preference shown by postlarvae as a useful adaptation to their natural habitats. Similarly, higher growth rates were reported at low salinities for *P. vannamei* (Bray *et al.*, 1994; Samocha *et al.*, 1998), *P. monodon* (Rajyalakshmi and Chandra, 1987), *P. semisulcatus* (Harpaz and Karplus, 1991) and *M. dobsoni (*Kuttyamma, 1982). In *P. merguiensis*, Staples and Heales (1991) reported maximum growth at 31^0C and 30‰, while survival was highest at 20^0C and 20‰. Varghese (1999) reported high survival in *P. indicus* postlarvae at high salinity, while growth was greater at low salinity.

On the contrary, reports of reduced growth rate of penaeid shrimps at lower salinities also exist. Lester and Pante (1991) pointed out that salinities below 5‰ and above 35‰ usually have negative effects on shrimp growth although in *P. monodon* good growth and survival was reported in freshwater (Pantastico, 1979). Low growth was reported in *P. monodon* (Navas and Sebastian, 1989), *P. semisulcatus* (Harpaz and Karplus, 1991) and *P. indicus* (Vijayan and Diwan, 1995) at salinities below 5‰. Cawthrone *et al.* (1983) observed good growth and

survival of *P. monodon* in full strength seawater than lower salinity. Similar observation was made by Ponce-Palafox *et al.* (1997) in *P. vannamei* where survival and growth coincided best at around 28-30^{0}C and 33-40‰. Similarly, in *Litopenaeus setiferus* maximum growth was reported at 40‰ while low growth rate was obtained at 10‰ for PL10-15 (Rosas *et al.*, 1999). O'Brien (1994) found that *P. esculentus* grew fast at 30^{0}C and 30‰ while its survival rate was maximum at 24^{0}C and 34‰. Allan and Magiure (1992) observed no significant difference in growth of *P. monodon* between 15 and 30‰. These observations show that although penaeids survive in a wide range of salinities, best growth increment takes place only in the optimum range (10-30‰) (Kinne, 1970a, b).

In comparison to the commercial species of penaeids very little is known about the growth of *P. merguiensis*. Growth experiments on *P. merguiensis* have been limited to studies of their nutritional requirements (Beard *et al.*, 1977; Sedgewick, 1979, 1980; Sadhana and Neelakantan, 1996, 1997; Thongrod and Boonyaratpalin, 1998), while some pond trials have been reported (Gundermann and Popper, 1975; Hariati *et al.*, 1998). Vinod *et al.* (1996) studied the effect of salinity on growth, food intake and conversion efficiencies of juvenile *P. merguiensis* and 20‰ was reported to be optimum at which the food consumption and production was high. Growth and productivity of juvenile *P. merguiensis* in natural and laboratory systems were studied by Staples *et al.* (1984). A thorough scrutiny of available literature suggests that the studies conducted on the effect of salinity on growth and survivals of *P. merguiensis* are not conclusive. In the tropics, as temperature does not have a decisive role in growth and survival of postlarvae, the present set of experiments were conducted with the objective to understand the effect of various salinities on growth and survival in the postlarval stages of *P. merguiensis*.

MATERIALS AND METHODS

Experimental condition

Postlarvae of *P. merguiensis* were collected from Kali Estuary, North Karnataka with a 2mm mesh seine net and transported to the laboratory in oxygenated bags. On reaching the laboratory they were segregated and postlarvae of almost the same length range were used for the present experiments. Growth studies were conducted in plastic tubs, each with an effective water volume of forty litres. Aeration was provided by a single airstone to all treatments (Plate 22).

Survival and growth rate were studied in triplicate conditions under four salinity ranges (5, 15, 25 and 35‰) for four weeks. Groups of 25 animals were initially distributed into the tubs filled with seawater. From the initial salinity of 20‰, the young shrimps were acclimated to the required salinity levels by diluting the seawater in the case of lower saline treatments and by adding brine in case of higher saline treatments. As in the earlier experiments desired salinity was obtained by mixing seawater with freshwater using the formula

$$V = \frac{\text{Desired salinity}}{\text{Salinity of seawater}} \times 100$$

where V is the volume of seawater of known salinity. The salinity was changed at the rate of 5‰/day. Acclimation to all the treatments was achieved within four days. During the experimental period the shrimps were fed *ad libitum* with commercial shrimp feeds (C.P.Feed, with 37% protein). Faeces and uneaten food were siphoned out from the containers and thirty percent of the water was replaced daily before adding feed.

PLATE 22. Part of the experimental set up used to study the effect of salinity on postlarval growth

Effect of salinity on survival and growth of postlarvae exposed to different salinity

Initial measurements in each experiment were obtained from samples of ten postlarvae of shrimps withdrawn from the source population. The animals were observed daily and a random sample of ten animals were taken out from each treatment for weekly measurements of total length, while weights were measured fortnightly. After excess of water was removed, the length of each specimen was measured to the nearest 0.5mm and weight determined to the nearest 0.1mg. Both the largest and the smallest specimen in each tank were included in order to determine size ranges. At the termination of the experiment the total number, average weight and length of the surviving shrimps in each tank were noted. Growth was obtained from the difference in weight and length before and after exposure to each of the experimental salinities. Specific growth rates (SGR) were calculated using the formula

$$SGR = \frac{\text{Ln (Final wet body weight) - Ln (initial wet body weight) x 100}}{\text{Time taken (days)}}$$

A performance index (PI) (Rosas *et al.*, 1999) was calculated to establish the combined effect of salinity on growth and survival of postlarva, and thus integrate both responses. This index was calculated as follows:

PI = Growth rate (mg/ day) x Survival (%)

The data were subjected to one-way ANOVA to establish differences between treatments. Differences were considered significant if $P< 0.05$.

RESULTS

Survival of postlarvae: The survival and growth of postlarvae in different salinities are given in Table 18. Figure 7 displays the survival of postlarvae of *P. merguiensis* after subjecting to various salinities for a period of 28 days. From the results obtained a high survival of postlarvae was obtained at 25‰ (80%) while the lowest survival rate was recorded at 5‰ (22%). Intermediate survival rates were obtained at 15 and 35‰ being 61.5 and 43.0% respectively. From table 18 it was clear that a survival of more than 50% was obtained in salinity ranges from 15‰- 25‰.

Growth trials on postlarvae: Although *P. merguiensis* can grow in a wide range of salinities by virtue of their euryhaline nature, the most favourable growth increment was recorded at 15‰ as shown in Table 18.

Pronounced differences in the growth rates were visible from the second week onwards. The growth of *P. merguiensis* in terms of length was highest 15‰ after a period of 14 days (0.91mm/day) and also after 28 days of observation (0.57mm/day), while the lowest increase in growth rate was observed at 35‰. The average daily gain in length after a period of 28 days was the highest at 15‰ being 0.57mm/day followed by 5‰ (0.51mm/day).

Similarly, growth in terms of wet weight was also highest at 15‰ after a period of 14 days (1.66mg/day) and also after 28 days of observation (2.89mg/day), while the lowest increase in growth rate was observed at 35%. The average daily gain in weight per day was also highest in 15‰ (2.89mg/day). This was also confirmed by analysis of specific growth rate of postlarvae reared at different salinities. A maximum specific growth rate was obtained at 15‰, while the lowest value was obtained at 35‰ (Table 18, Figure 8).

FIGURE 7: Survival of *Penaeus merguiensis* postlarvae at different salinities

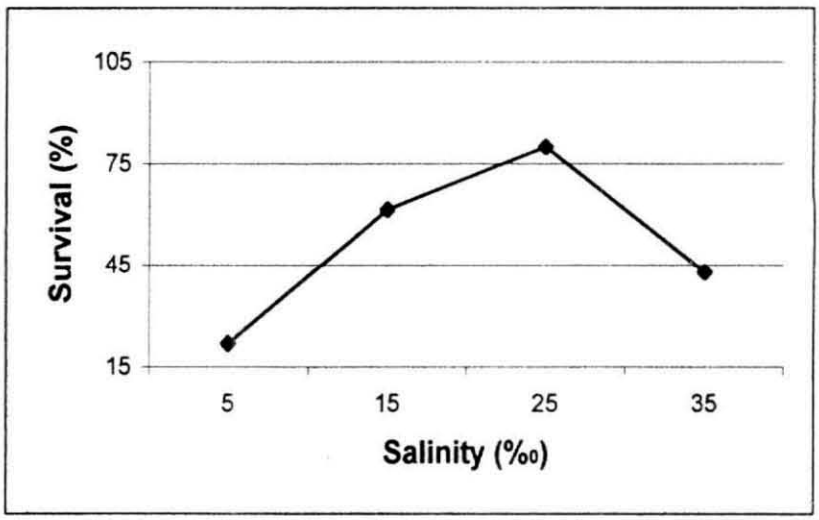
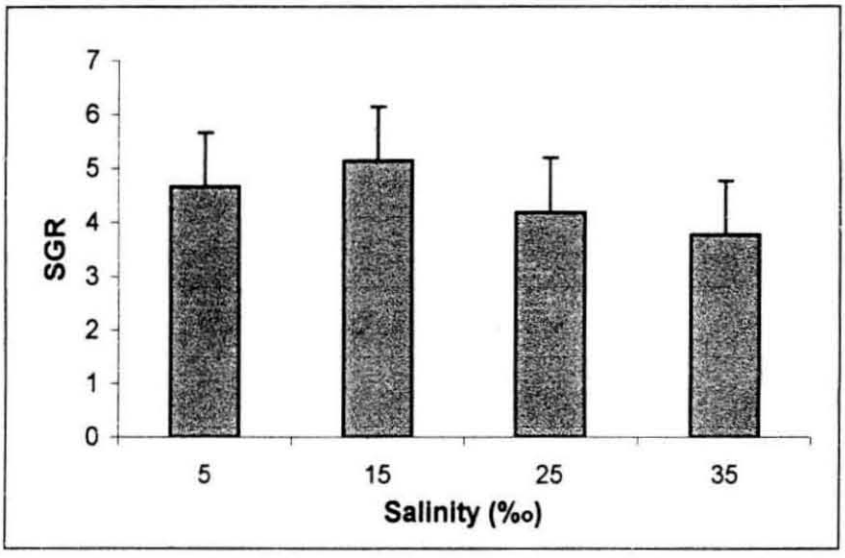


Table 18: Experimental values for growth and survival in the growth experiment of *P. merguiensis*

Characters observed	Test salinities			
	5ppt	**15ppt**	**25ppt**	**35ppt**
Av. Initial lt. (mm)	9.23	9.17	9.3	9.2
Av. Final lt(mm)	23.43	25.03	21.37	20.43
Av. Initial wt. (mg)	3.21	3.25	2.76	2.84
Av. Final wt(mg)	61.33	84	45	34.67
Stocking density(nos/m^2)	25	25	25	25
Survival percentage	22	61.5	80	43
Growth rate (mm/ day)				
14 days	0.84	0.91	0.76	0.73
28 days	0.51	0.57	0.43	0.40
Growth rate(mg/ day)				
14 days	1.19	1.66	0.93	0.59
28 days	2.08	2.89	1.48	1.13
Av. Daily gain (mm/ day)	0.51	0.57	0.43	0.40
Av. Daily gain (mg/ day)	2.08	2.89	1.48	1.13
Specific growth rate	4.67	5.15	4.19	3.77
Performance index	45.8	177.8	118.08	48.55

FIGURE 8. Specific growth rate of *Penaeus merguiensis* postlarvae reared at different salinity

Vertical bars indicate standard deviation



From the table, it can be clearly seen that the growth was different at various salinities tested. Thus, postlarvae of *P. merguiensis* maintained at a salinity level of 15% exhibited a higher growth rate compared to all other tested salinities. Growth rates were found to decrease with increase in salinity to 25 and 35‰. Maximum values for performance index were also obtained at 15‰ (177. 797) while the lowest values was obtained at 5‰ (45.8) (Table 18, Figure 9).

ANOVA (Table 19 and 20) indicated that salinity had a significant effect ($p < 0.05$) on growth of postlarvae of *P. merguiensis*.

DISCUSSION

The experiments conducted on the postlarval stages of *P. merguiensis* illustrated the well-developed euryhaline character of this species. The results of the experiments provided a convincing evidence for the important role of salinity in improving the growth performance of the postlarval stages of *P. merguiensis*. In the present study, the postlarvae of *P. merguiensis* collected and acclimated to the test salinities of lower and higher salinities showed varying results in their survival rate. The survival rate was the highest at 25‰ followed by in 15‰.

The growth experiments conducted on *P. merguiensis* for a period of 28 days, revealed that growth in terms of increase in length and weight changed with variation in salinity of the medium. Growth is the manifestation of the net outcome of energy gains and losses within a framework of abiotic and biotic conditions (Brett and Grovers, 1979). The present result showed that the growth of *P. merguiensis* at 35‰ was severely arrested. Maximum growth in terms of length was obtained at a lower salinity of 15‰ after 28 days. It was relatively low at 25 and 35‰. Similarly, increase in growth in terms of wet weight was also highest at 15‰ with specific growth rate being 5.15. This result corroborates the fact that

FIGURE 9: Performance index of postlarvae of *Penaeus merguie* at different salinities

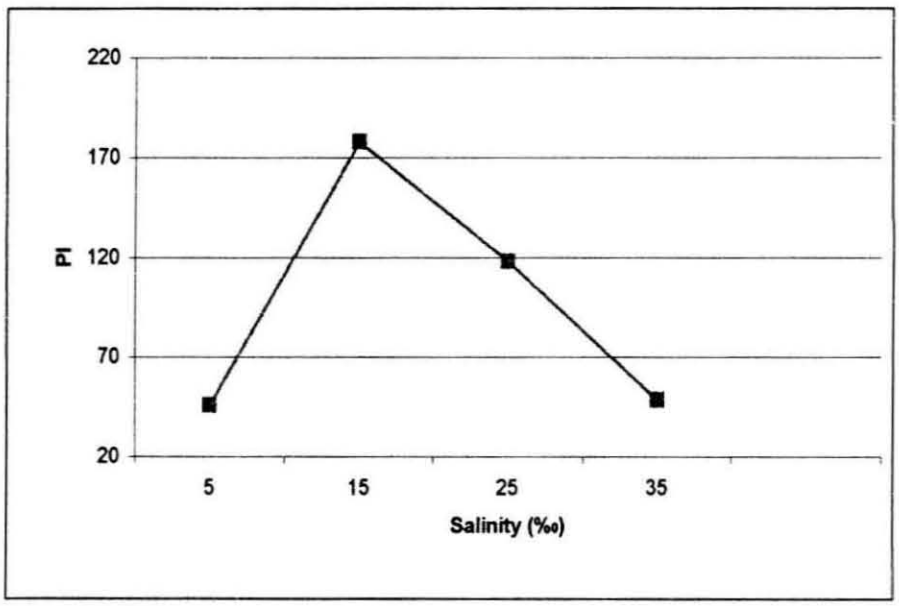


Table 19: ANOVA of growth rate in lengtht of postlarvae of *Penaeus merguiensis* reared at different salinities

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	0.050	3.000	0.017	5.017	0.030
Within Groups	0.027	8.000	0.003		
Total	0.077	11.000			

Table 20: ANOVA of growth rate in wet weight of postlarvae of *Penaeus merguiensis* reared at different salinities

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	5.311	3.000	1.770	13.438	0.002
Within Groups	1.054	8.000	0.132		
Total	6.365	11.000			

better growth is obtainable at lower salinity levels as also reported by Harpaz and Karplus (1991) for *P. semisulcatus* and Deshimaru *et al.* (1985) for *P. monodon*.

Although growth can be an indicator of the physiological state of larvae it may not adequately reflect a particular condition if it is not accompanied by other indices of the general state of the organism in the culture tank. Thus, even if it is possible to obtain larger animals in a particular condition, it does not necessarily mean that there are more postlarvae in the harvest. Thus, Performance index was selected as a parameter to group all indices in one, by which the optimum condition of production was defined with more precision. The salinity condition of 15‰ was found to represent the optimum condition for production of *P. merguiensis* postlarvae based on this index. Zein- Eldrin (1963) reported salinity to have no affect on growth, which was contradicted by Gunter *et al.* (1964) and also by the present study as better performance index were obtained at 15‰.

The preference of younger penaeids for low saline waters and also reports of their good growth and survival at lower salinity levels have been demonstrated for several species (Gunter, 1961; Gunter *et al.*, 1964; Venkataramaiah *et al.*, 1972; Nair *et al.*, 1975; Gopalakrishnan, 1976; Dall, 1981, Kuttyamma, 1982; Raj and Raj, 1982; Harpaz and Karplus, 1991; Staples and Heales, 1991; Bray *et al.*, 1994; Vijayan and Diwan, 1995; Samocha *et al.*, 1998). In many cases this was also found to agree with the habitat preference of the species in natural environments (Selvakumar *et al.*, 1977; Staples, 1980; Achuthankutty and Parulekar, 1986; Staples and Vance, 1987; Gunaga *et al.*, 1989). The results of the present study indicated that postlarvae of *P. merguiensis*, like many other penaeid shrimps, do well in seawater of lower salinity.

The temperature in the present study ranged between 28-30^{0}C, the normal temperature found in most brackishwater nursery grounds of *P. merguiensis* along Indian coast. This temperature being characteristic of tropical

region and its greater stability in the inshore and estuarine regions makes it possible for the young ones of this species to withstand wide range of salinity. This is in support of the view of Bhattacharya and Kewalramani (1976) that temperature does not play a significant role in the occurrence of postlarvae in Indian estuarine and brackishwater environments.

Salinity is thought to act on two independent aspects of physiology *viz*., growth and osmoregulation. It has been hypothesised that osmoregulatory costs are lowest in an iso-osmotic environment and that the consequent energy savings permit increased growth. Outside the optimum range, the growth increment attained was comparatively low. In the extreme range of salinities, shrimps must expend considerable energy for osmoregulation at the expense of other processes, such as growth. Despite the high osmoregulatory capability of postlarvae of *P. merguiensis*, the results of the growth trial indicated that salinity had a significant effect on the survival and growth performance of this species as at a salinity of 5‰ and 35‰ growth was significantly impaired. This reduction in growth may be due to the increase in the energetic cost of osmoregulation. The superior growth rate at 15‰ may also be attributed to the better efficiency of consumption and utilisation of food and may also be due to the consistent growth associated with higher moulting frequency.

Staples and Heales (1991) suggested that optimum salinities, which are species- specific, reflect adaptation to the environment in which the animal is commonly found. In *P. merguiensis* postlarvae, lower salinity of 15‰ can be defined as the optimum salinity since these shrimps grow equally well at this salinity while a salinity range of 15 - 25‰ can be defined as the optimum salinity range as growth and survival was found to be comparable within this range.

Of the many penaeid shrimps that penetrate estuaries, *P. merguiensis* seems to be a natural candidate for rearing in waters of low salinity. The study clearly indicates a high coincidence between the experimentally

determined optimum conditions for production and prevailing conditions in which the animal spends its postlarval and juvenile phases (Goswami and George, 1978; Goswami and Goswami, 1992; Parulekar and Achuthankutty, 1993; Ronquillo and Saisho, 1993) and hence explains why these shrimps perform well at lower salinity. By extrapolating the laboratory results, it could be predicted that postlarval shrimps moving into estuary when salinities are optimum would grow quickly with minimum mortality. Therefore, to achieve high productivity in aquaculture of this species, a compromise between the optimum conditions for growth and survival must be reached. As optimum biomass was obtained at 15‰, ponds with salinity levels around these values all year would provide ideal conditions.

SUMMARY

SUMMARY

The present study was attempted to understand the fundamentals of various aspects of ovarian maturation and larval development of *Penaeus merguiensis* De Man and also to investigate the response of females of the species to various induced maturation techniques generally employed in different hatcheries. The salient and significant findings are listed below.

1. The general structure of female reproductive system appears to be homologous with other penaeids with paired ovaries, oviducts and a single thelycum.

2. The ovarian maturity stages in *P. merguiensis* were classified into five stages *viz.* Previtellogenic, early vitellogenic, late vitellogenic, vitellogenic and spent.

3. Anatomical observation revealed the change in colour of ovary from pale buff or yellow to dark green with corresponding increase in GSI and ova diameter as maturation progressed.

4. Oogenesis followed the typical pattern as in other penaeids with development of cortical bodies in the vitellogenic stage, increase in ova diameter and appearance of oil globules in cytoplasm with advancement in maturation.

5. Native PAG Electrophoretic analysis of serum revealed the presence of vitellogenin (MW 350kDa) in vitellogenic females with two corresponding vitellins (MW 300 and 550kDa) in the ovary.

6. Both vitellogenin and vitellin have been characterised as lipoglycoproteins with calcium affinity.

7. SDS PAGE analysis conducted on vitellogenin and vitellin revealed the presence of six polypeptide subunits in vitellogenin (molecular weights - 104, 102, 99, 68, 64 and 24kDa) and five subunits in vitellin (molecular weights - 104, 102, 68, 55 and 44kDa).

8. Eyestalk ablation in *P. merguiensis* collected from brackishwater shrimp culture ponds and maintained in units set out in the bay resulted in ovarian maturation within 3-5 days, while there was no effect on maturation in females of smaller size maintained under the same environmental condition.

9. Ablating females during the colder months of the year resulted in delayed response indicating the effect of season on the reproductive performance of broodstock of *P. merguiensis*.

10. Alternative methods to eyestalk ablation, like administration of cerebral and thoracic ganglionic extracts, injection of progesterone and 5- hydroxytryptamine were tried to induce ovarian maturation. These treatments resulted in an increase in GSI indicating that maturation was in progress. Among these 5-HT treatment gave comparatively better results

11. General pattern of larval development in *P. merguiensis* was similar to that of other penaeids with six naupliar stages followed by three protozoeal stages and three mysis stages before the first postlarval stage.

12. Effect of different salinity and temperature on hatching percentage of *P. merguiensis* eggs revealed that the success rates were highest at the 35‰ and 33 ± 0.5^{0}C.

13. A salinity-temperature combination of 35‰ and 33 ± 0.5^{0}C resulted higher survival rates during the naupliar stages.

14. Survival of protozoea (PZ1) to postlarvae (PL1) was also highest at 35‰ and 33 ± 0.5^{0}C. Maximum growth was also obtained at this condition as indicated by the high MSI values.

15. Survival and growth experiments conducted on postlarvae maintained at different salinities revealed that the best results were obtained at 15‰.

From the present study, it has been proved that brackishwater farm-raised shrimps of *P. merguiensis* could be matured within three to five days in captivity by subjecting them to eyestalk ablation and maintaining them under marine conditions. Alternatives to eyestalk ablation tried in the present study revealed that 5-hydroxytryptamine to stimulate ovarian maturation in *P. merguiensis* at a faster rate suggesting it to be a practical alternative to eyestalk ablation. The experiments conducted on larval stages of *P. merguiensis* showed that marine conditions (35‰) and higher temperature (33 ± 0.5^{0}C) are optimum for the early larval developmental stages. The postlarval stages of this species were found to exhibit better survival and faster growth rate at 15‰. Therefore, to achieve high productivity in the aquaculture of this species, a salinity of 15‰ appears to be optimum. Thus, the present study suggests *P. merguiensis* to be an alternative to *P. monodon* in areas where it forms a dominant species

REFERENCES

REFERENCES

Abdu, U., Yehezkel, G. and Sagi, A., 2000. Oocyte development and polypeptide dynamics during ovarian maturation in the red-claw crayfish *Cherax quadricarinatus*. *Inv. Rep. Dev.*, 37 (1): 75 - 83.

Achuthankutty, C. T. and Parulekar, A. H., 1986. Biology of commercially important penaeid prawns of Goa waters. *Ind. J. Mar. Sci.*, 15: 171 - 173.

Adiyodi, R. G., 1968. Protein metabolism in relation to reproduction and moulting in the crab, *Paratelphusa hydrodromous* (Herbst): Part I - Electrophoretic studies on the mode of utilization of soluble proteins during vitellogenesis. *Indian J. Exp. Biol.*, 6: 144 - 147.

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

Adiyodi, R. G. and Subramoniam, T., 1983. Arthropoda- Crustacea (Oogenesis, Oviposition and Oosorbtion). *In* : Reproductive Biology of Crustacea Vol. 1. Oogenesis, Oviposition and Oosorbtion (ed. Adiyodi, K. G.). Wiley, London, pp. 443 - 495.

Adiyodi, R. G., 1985. Reproduction and its control. *In*: The Biology of Crustacea, Vol. 9. (ed. Bliss, D. E. and Mantel, L. H). Academic Press, New York, pp. 147 - 215.

Alikunhi, K. H., Poernomo, A., Adisukresno, S., Budiono, M. and Busman, S., 1975. Preliminary observations on induction of maturation and spawning in *Penaeus monodon* Fabricius and *Penaeus merguiensis* de Man by eyestalk extirpation. *Bull. Shr. Cult. Res. Cent.*, 1: 1 - 11.

Allan, G. L. and Magiure, G. B., 1992. Effect of pH and salinity on survival, growth and osmoregulation in *Penaeus monodon*. *Aquaculture*, 107: 33 - 47.

Anderson, W. W., King, J. E. and Lindner, M. J., 1949. Early stages in the life history of the common shrimp *Penaeus setiferus* (Linnaeus). *Biol. Bull.*, 96: 168 - 172.

Anderson, S.L., Chang, E. S. and Clark, W. H., Jr., 1984. Timing of postvitellogenic ovarian changes in the ridgeback prawn *Sicyonia ingentis* (Penaeidae) determined by ovarian biopsy. *Aquaculture*, 42: 257 - 271.

AQUACOP, 1975. Maturation and spawning in captivity of penaeid shrimp: *Penaeus merguiensis* de Man, *P. japonicus* Bate, *P. aztecus* Ives, *Metapenaeus ensis* de Hann and *P. semisulcatus* de Haan. Proc. World Maricul. Soc., 6: 123 - 132.

AQUACOP, 1977a. Observations on the maturation and reproduction of penaeid shrimp in captivity in a tropical medium. *Aquaculture Workshop, ICES*. 10 - 13 May. 1977. Brest, France, pp. 34

AQUACOP, 1977b. Reproduction in captivity and growth of *Penaeus monodon* in Polynesia. Proc. World Maricul. Soc., 8: 927 - 945.

AQUACOP, 1979. Penaeid reared broodstock: closing the cycle of *Penaeus monodon*, *P. stylirostris* and *P. vannamei*. Proc. World Maricul. Soc., 10: 445 - 452.

Arechiga, H., Banuelos, E., Frixione, E., Picones, A. and Rodriguez-Sosa, L., 1990. Modulation of crayfish retinal sensitivity by 5-hydroxytryptamine. *J. Exp. Biol.*, 150: 123 - 143.

Arnstein, D. R. and Beard, T. W., 1975. Induced maturation of the prawn *Penaeus orientalis* Kishinouye in the laboratory by means of eyestalk ablation. *Aquaculture*, 5: 411 - 412.

Bakker-Grunwald, T. and Applebaum, S. W., 1977. A quantitative description of vitellogenesis in *Locusta migratoria migratorioides*. *J. Insect. Physiol.*, 23: 259 - 263.

Bas, C. C. and Spirak, E. D., 2000. Effect of salinity on embryo of two Southeastern Atlantic Estuarine Grapsid crab species cultured in vitro. *J. Crust. Biol.*, 20(4): 647 - 656.

Beard, T. W., Wickens, J. F. and Arnstein, D. R., 1977. The breeding and growth of *Penaeus merguiensis* de Man in laboratory recirculating systems. *Aquaculture*, 10: 275 - 289.

Bell, T. A. and Lightner, D. V., 1988. A handbook of normal penaeid shrimp histology. World Aquacult. Soc., Baton Rouge, Louisiana, pp. 1 - 107.

Bhattacharya, S. S. and Kewalramani, G., 1976. Salinity and temperature tolerances of *Acartina spinicauda*. *Ind. J. Mar. Sci.*, 5: 28 - 30.

Bomirski, A. and Kleke, E., 1974. Action of eyestalks on the ovary in *Rhithropanopeus harrisii* and *Crangon crangon* (Crustacea: Decapoda). *Mar. Biol.*, 24: 329 - 337.

Bomirski, A. and Klek - Kawinska, E. K., 1976. Stimulation of oogenesis in the sand shrimp, *Crangon crangon*, by a human gonadotropin. *Gen. Comp. Endocrinol.*, 30: 239 - 242.

Bose, K. S., 1995. Studies on the reproductive physiology of the green tiger prawn *Penaeus (Penaeus) semisulcatus* (de Haan). Ph. D. Thesis, Cochin University of Science and Technology.

Bradfield, J. Y., Berlin, R. L., Rankin, S. M and Keeley, L. C., 1989. Cloned cDNA and antibody for an ovarian cortical granule polypeptide of the shrimp *Penaeus vannamei*. ***Bio. Bull.,*** 177(3): 344 - 349.

Bray, W. A. and Lawrence, A. L., 1992. Reproduction of *Penaeus* species in captivity. *In* : Marine shrimp culture: Principles and Practices (ed. Fast, A. W. and Lester, L. J.). Elsevier, Amsterdam, pp. 93 - 170.

Bray, W. A., Lawrence, A. L. and Leung-Trujillo, J. R., 1994. The effect of salinity on growth and survival of *Penaeus vannamei*, with observations on the interaction of IHHN virus and salinity. *Aquaculture*, 122: 133 - 146.

Brett, J. R. and Grovers, T. D. D., 1979. Physiological energetics. *In:* Fish physiology, Vol. 8 (ed. Hoar, W. S. and Randell, D. J.). Academic Press, New York, pp. 279 - 352.

Browdy, C. L. and Samocha, T. M., 1985a. Maturation and spawning of ablated and nonablated *Penaeus semisulcatus* de Haan, 1844. *J. World Aquacul. Soc.*, 16: 236 - 249.

Browdy, C. L. and Samocha, T. M., 1985b. The effect of eyestalk ablation on spawning, moulting and mating of *Penaeus semisulcatus* de Haan. *Aquaculture*, 59: 251 - 258.

Browdy, C. L., Hadini, A., Samocha ,T. M. and Loya, Y.,1986. The reproductive performance of wild and pond-reared *Penaeus semisulcatus* de Haan. *Aquaculture*, 59: 251 - 258.

Browdy, C. L., Fainzilber, M., Tom, M., Loya, Y. and Lubzens, E., 1990. Vitellin synthesis in relation to oogenesis in *in vitro* incubated ovaries of *Penaeus semisulcatus* (Crustacea; Decapoda; Penaeidae). *J. Exp. Zool.*, 255: 205 - 215.

Browdy, C., 1992. A review of the reproduction biology of *Penaeus* species: perspectives on controlled shrimp maturation for high quality nauplii production. *In:* Proceedings of the Special Session on Shrimp Farming. (ed. Wyban, J.). World Aquaculture Society, Baton Rouge, LA, pp. 22 - 51.

Browdy, C., 1998. Recent developments in penaeid broodstock and seed production technologies improving the outlook for superior captive stocks. *Aquaculture*, 164 (1-4): 3 - 21.

Brown, A., McVey, J. P., Middleditch, B. S. and Lawrence, A. L., 1979. Maturation of white shrimp (*Penaeus setiferus*) in captivity. Proc. World Maricul. Soc., 10: 435 - 444.

Bun, N. T. and Idler, D. R., 1983. Yolk formation and differentiation in teleost fish. *In:* Fish Physiology, Vol. 4A (ed. Hoar, W. S. and Randall, D. J.). Academic Press, New York, pp. 373 - 397.

Byard, E. H. and Aiken, D. E., 1984. The relationship between moulting and reproduction and a haemolymph female specific protein in the lobster *Homarus americanus*. *Comp. Biochem. Physiol.*, 77A (4): 749 - 758.

Caillouet, C. W., 1972. Ovarian maturation induced by eyestalk ablation in pink shrimp, *Penaeus duorarum* Burkenroad. Proc. World Maricul. Soc., 3: 205-255.

Cao, S., Xiang, B. and Fang, H., 1990. Evaluation of some phytoplankton as food for zoea of *Penaeus orientalis*. *Mar. Sci. Bull.*, 9(3): 55 - 62.

Cardona, H. B. and Capo, T. K., 1992. Maturation and spawning in captivity of *Penaeus duorarum* using eyestalk ablation. *J. Shellfish Res.*, 11(1): 191 - 192.

Cavalli, R. O., Scardua, M. P. and Wasielesky, W. Jr., 1997. Reproductive performance of wild and pond reared *Penaeus paulensis* females. *J. World. Aquacult. Soc.*, 28: 260 - 267

Cawthrone, D., Beard, F., Davenport, J. and Wickins, J. F., 1983. Responses of juvenile *Penaeus monodon* Fabricius to natural and artificial seawater of low salinity. *Aquaculture*, 32: 165 - 174.

Chakraborti, D. L., Bowmik, M. L. and Halder, D. D., 1986a. Effect of change in salinity on the survival of *Penaeus monodon* postlarvae. *Ind. J. Fish.*, 33(4): 484 - 487.

Chakraborti, D. L., Halder, D. D., Das, S. K., Mandal, S. and Bowmik, M. L., 1986b. Growth of *Penaeus monodon* Fabricius under different environmental conditions. *Aquaculture*, 51: 189 - 194.

Chamberlain, G. W. and Lawrence, A. L., 1981. Maturation, reproduction and growth of *Penaeus vannamei* fed natural diets. *J. World Maricul. Soc.*, 12: 209 - 224.

Chamberlain, G. W. and Gervais, N. F., 1984. Comparison of unilateral eyestalk ablation with environmental control for ovarian maturation. *J. World Maricult. Soc.*, 15: 29 - 30.

Chan, H. H. and Lim, S. H., 1988. Effects of progesterone on the ovarian maturation of the white prawn *Penaeus merguiensis* de Man. *In:* Aquaculture International Congress and Exposition, Vancouver. Trade and Convention centre, Vancouver, British Columbia, Canada, pp. 63.

Chandran, M. R., 1968. Studies in the marine crab, *Charbydis variegata*. 1. Reproductive and Nutritional cycle in relation to breeding periodicities. Proc. Indian Acad, Sci., 67: 215 - 223.

Chang. C. F., Lee, F. Y. and Huang, Y. S., 1993a. Purification and characterization of vitellin from the mature ovaries of prawn, *Penaeus monodon*. *Comp. Biochem. Physiol.*, 105B: 409 - 414.

Chang, C. F., Shih, T. W. and Hong, T. H., 1993b. Purification and characterization of vitellin from the mature ovaries of prawn, *Macrobrachium rosenbergii*. *Comp. Biochem. Physiol.*, 105B: 609 - 615.

Chang, C. F., Lee, F. Y., Huang, Y. S. and Hong, T. H., 1994. Purification and characterization of the female specific protein (vitellogenin) in mature female haemolymph of the prawn *Penaeus monodon*. *Invert. Reprod. Dev.*, 25: 185 - 192.

Chang, C. F. and Jeng, S. R.,1995a. Purification and characterization of female specific protein (vitellogenin) in mature female haemolymph of the prawn, *Penaeus chinensis*. *Comp. Biochem. Physiol.*, 112B: 257 - 263.

Chang, C. F. and Jeng, S. R.,1995b. Purification and characterization of vitellin from the ovaries of the prawn, *Penaeus chinensis*. *Invert. Reprod. Dev.*, 29: 87 - 93.

Chang. C. and Shih T., 1995. Reproductive cycle of ovarian development and vitellogenin profiles in the freshwater prawns, *Macrobrachium rosenbergii*. *Invert. Reprod. Dev.*, 27(1): 11 - 20.

Chang. F. C., Jeng. S. R., Lin. M. N. and Tin, Y. Y., 1996. Purification and characterisation of vitellin from the mature ovaries of prawn, *Penaeus chinensis*. *Invert. Reprod. Dev.*, 29(2): 87 - 93.

Charmantier-Daures, M., Thuet, P., Charmantier, G. and Trilles, J. P., 1988. Tolerance a la salinite et osmoregulation chez les post-larves de *Penaeus japonicus* et *P. chinensis*. Effect de la temperature. *Aquat. Living Resour.*, 1: 267 - 276.

Charmantier, G., Charmantier-Daures, M. and Van Herp, F., 1997. Hormonal regulation of growth and reproduction in crustaceans. *In:* Recent Advances in Marine Biotechnology (ed. Fingerman, M., Nagabhushanam, R. and Thompson, M. F.). Oxford and IBH , New Delhi, pp. 109 - 161.

Charmantier, G., 1998. Ontogeny of osmoregulation in crustaceans: a review. *Invert. Reprod. Dev.*, 33 (2 - 3): 177 - 180.

* Charniaux-Cotton. H., 1975. Control hormonale de la differenciation sexuella etde le activite genitale chez les crustace, Malocastraces. Publ. Stz. Zool. Napoli, 139: 480 - 509.

* Charniaux-Cotton, H., 1985. Vitellogenesis and its control in malacostracan Crustacea. *Am. Zool.*, 25: 197 - 205.

Charniaux-Cotton, H. and Payen, G. G., 1988. Crustacean reproduction. *In:* Endocrinology of Selected Invertebrate Type, Vol. 2. (ed. Laufer, H. and Downer, R. G. H.). Alan R. Liss, New York, pp. 279 - 303.

Chen, C. C. and Chen, S., 1993. Isolation and partial characterization of vitellin from the egg of the giant tiger prawn, *Penaeus monodon*. *Comp. Biochem. Physiol.*, 106B: 141 - 146.

Chen, C. C. and Chen, S., 1994. Vitellogenesis in the giant tiger prawn, *Penaeus monodon* Fabricius, 1798. *Comp. Biochem. Physiol.*, 106 (B): 141 - 146.

Chong, V., 1991. The larval stages of the Malaysian penaeid shrimp (Trachypenaeus *fulvus* Dall, 1957) reared in the laboratory. *Asian Fish. Sci.*, 4(2): 165 - 187.

Choo, P. S., 1987. Effects of salinity on the spawning, egg-incubation and larviculture of *P.merguiensis*. *Fish. Bull.*, 52:9 – 12.

Chow, S., Browdy, C. L. and Sandifer, P. A., 1993. Timing of ovulation of the Atlantic white shrimp *Penaeus setiferus* (Linnaeus, 1767) (Decapoda, Penaeidae). *Crustaceana*, 64(2): 242 - 248.

Choy, S. C., 1987. Growth and reproduction of eyestalk ablated *Penaeus canaliculatus* (Olivier, 1811) (Crustacea: Penaeidae). *J. Exp. Mar. Biol. Ecol.*, 112(2): 93 - 107.

Chu, K. H., 1989. *Chaetoceros gracilis* as the exclusive feed for the larvae and postlarvae of the shrimp *Metapenaeus ensis*. *Aquaculture*, 89 (3 - 4): 81 - 87.

Clark, W. H. and Lynn, J. W., 1977. A Mg^{2+} dependent cortical reaction in the eggs of penaeid shrimp. *J. Exp. Zool.*, 200: 177 - 181.

Clark, J. W. Jr., Yudin, A. I., Lynn, K. W, Griffin, F. J. and Pillai, M. C., 1990. Jelly layer formation in penaeoidean shrimp eggs. *Biol. Bull.*, 178: 295 - 299.

Cook, H. L. and Murphy, M. A., 1969. The culture of larval penaeid shrimp. *Trans. Am. Fish. Soc.*, 98: 751 - 754.

Cook, H. L. and Lindner, M. J., 1970. Synopsis of biological data on brown shrimp, *Penaeus aztecus* Ives, 1891. FAO Fish. Rep., 57(4): 1471 - 1497.

Costlow, J. D. Jr., Bookhout, C. G. and Monroe, R., 1960. The effect of salinity and temperature on larval development of *Sesarma cinereum* (Bose) reared in the laboratory. *Biol. Bull.*, 118: 183 - 202.

Crim, L. W., Chang, J. P. and Peter, R. E., 1984. Effects of dopamine and apomorphine on gonadotropin release from the transplanted pars distalis in goldfish. *Gen. Comp. Endocrinol.*, 55: 347 - 350.

Crocos, P. J. and Kerr, J. D., 1983. Maturation and spawning of the banana prawn *Penaeus merguiensis* de Man (Crustacea: Penaeidae) in the Gulf of Carpentaria, Australia. *J. Exp. Mar. Biol. Ecol.*, 69: 37 - 59.

Croisille, Y., Junera, H., Meusy, J. J. and Charniaux-Cotton, H., 1974. The female specific protein (Vitellogenic protein) in Crustacea with particular references to *Orchestia gammarella* (Amphipoda). *Am. Zool.*, 14: 1219 - 1228.

Croisille, Y. and Junera, H., 1980. Site of vitellogenin synthesis in *Orchestia gammarella* (Crustacea: Amphipoda). Immunohistological demonstration of appreciable amounts of vitellogenin in the subepidermal tissue of females in which secondary vitellogenesis is taking place. C. R. Hebd. Seances Acad. Sci. Ser. D., 290: 1487 - 1490.

Cummings, W. C., 1961. Maturation and spawning of the pink shrimp, *Penaeus duorarum* Burkenroad. *Trans. Am. Fish. Soc.*, 90: 462 - 468.

Dall, W., 1981. Osmoregulatory ability and juvenile habitat preference in some penaeid prawns. *J. Exp. Mar. Biol. Ecol.*, 55: 219 - 232.

Dall, W., Hill, B. J., Rothlisberg, P. C. and Staples, D. J., 1990. Reproduction. *In:* Adv. In Marine Biology, Vol. 27. The Biology of the Penaeidae (ed. Blaxter, J. H. S. and Southward, A. J.). Academic Press, London, U. K, pp. 251 -281.

Davis, B. J., 1964. Disc electrophoresis - II. Methods and application to human serum protein. Ann. N. Y. Acad. Sci., 121: 404 - 427.

De Kleijn, D. P. V. and Van - Herp, F., 1998. Involvement of hyperglycemic neurohormone family in the control of reproduction in decapod crustaceans. *Invert. Reprod. Dev.*, 33(2-3): 263 - 272.

Dehn, P. F., Aiken, P. E. and Waddy, S. L., 1983. Aspects of vitellogenesis in the lobster *Homarus americanus*. *Can. Tech. Rep. Fish. Aquat. Sci. D.*, 1161 (i -iv): 1 - 24.

Derelle, E., Grosclaude, J., Meusy, J. J., Junera, H. and Martin, M.,1986. ELISA titration of vitellogenin and vitelllin in the freshwater prawn, *Macrobrachium rosenbergii*, with monoclonal antibody. *Comp. Biochem. Physiol.*, 85B :1 - 4.

Deshimaru, O., Kuroki, D. K., Mazio ,M. A. and Kitamura ,S., 1985. Nutritional quality of compounded diets of prawn *Penaeus monodon*. *Bull. Jap. Soc. Sci. Fish.*, 51: 1037 - 1044.

Duerr, E. O., Molnar, A. and Sato, V., 1992. Microalgae as aquaculture feeds. *J. Phycol.*, 28(3): 12.

Duronslet, M. J., Yudin, A. I., Wheeler, R. S. and Clark, W. H., 1975. Light and fine structural studies of natural and artificially induced egg growth of penaeid shrimp. Proc. World Maricult. Soc., 6: 105 - 122.

Eastman - Reks, S. and Fingerman, 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(4): 679 - 684.

Eastman - Reks, S. and Fingerman, M., 1985. *In vitro* synthesis of vitellin by ovary of the fiddler crab, *Uca pugilator*. *J. Exp. Zool.*, 233: 111 - 116.

Elliott, R. H. and Gillot, G., 1979. An electrophoretic study of protein of the ovary, fat body and haemolymph in the migratory grasshopper *Melanoplus sanquinipes*. *J. Insect. Physiol.*, 25:405 - 410.

Elofsson, R., Laxmyr, L., Rosengren, E. and Hannson, C., 1982. Identification and quantitative measurements of biogenic amines and DOPA in the central nervous system and haemolymph of the crayfish *Pacifastacus leniusculus* *Crustaceana*, 71: 195 - 201.

Elofsson, R., 1983. 5-HT like immunoreactivity in the central nervous system of the crayfish, *Pacifastacus leniusculus*. *Cell Tiss. Res.*, 232: 221 - 236.

Emmerson, W. D., 1980. Induced maturation of prawn *P. indicius*. *Mar. Ecol. (Prog. Ser.)*., 2(2): 121 - 131.

Emmerson, W. D. and Andrew, B., 1981. The effect of stocking density on the growth, development and survival of *Penaeus indicus* Milne Edwards larvae. *Aquaculture*, 23: 45 - 57.

Emmerson, W. D., 1983. Maturation and growth of ablated and unablated *Penaeus monodon* Fabricius. *Aquaculture*, 32: 235 - 241.

Ewald, J. J., 1965. The laboratory rearing of the pink shrimp *Penaeus duorarum* (Burkenroad). *Bull. Mar. Sci.*, 15: 436 - 449.

Fainzilber, M., Tom, M., Shafir S., Applebaum, S. W. and Lubzens, E., 1992. Is there extra-ovarian synthesis of vitellogenin in penaeid shrimps? *Biol. Bull.*, 183: 233 - 241.

Fairs, N. J., Quinlan, P. T. and Goad, L. J., 1990. Changes in ovarian unconjugated and conjugated steroid titres during vitellogenesis in *Penaeus monodon*. *Aquaculture*, 89: 83 - 99.

FAO, 1997. Review of the state of World aquaculture. FAO Fisheries Circular No. 886, (Rev.1), pp. 163

FAO, 1999. Economic viability of marine capture fisheries. Findings of a global study and an interregional workshop. FAO Fish. Tec. Paper No. 377, Rome.

Fielder, D. R., Rao, K. K. and Fingerman, M., 1971. A female-linked lipoprotein and the diversity of haemocyanin components in the dimorphic variants of the fiddler crab, *Uca pugilator*, as revealed by disc electrophoresis. *Comp. Biochem. Physiol.*, 39B: 291 - 297.

Fingerman, M., Julian, W. E., Sprites, M. A. and Kostrzewa, R. M., 1974. The presence of 5-hydroxytryptamine in the eyestalks and brain of the fiddler crab *Uca pugilator*, its quantitative modification by pharmacological agents and possible role as a neurotransmitter in controlling the release of red pigment-dispersing hormone. *Comp. Gen. Pharmacol.*, 5: 299 - 303.

Fingerman, M., 1987. The endocrine mechanisms in crustaceans. *J. Crust. Biol.*, 7: 1 - 24.

Fingerman, M., 1997. Roles of neurotransmitters in regulating reproductive hormone release and gonadal maturation in decapod crustaceans. *Invert. Reprod. Dev.*, 31: 47 - 54.

Fry, F. E. J., 1971. The effect of environmental factors on the physiology of fish. *In:* Fish Physiology, Vol. VI, (ed. Hoar, W. S., Randall, J. D.). Academic Press, London, pp. 1 - 98.

Fyffe, W. E. and O'Connor, J. D., 1974. Characterization and quantification of a crustacean lipovitellin. *Comp. Biochem. Physiol.*, 47B: 851 - 867.

Garcia, S. and Le Reste, L., 1981. Life cycles, dynamics, exploitation and management of coastal penaeid shrimp stocks. FAO Fish. Tech. Pap., No.203, pp. 215.

Gomez, R., 1965. Accleration of development of gonads by implantation of brain in the crab, *Paratelphusa hydrodromous*. *Naturwissenschaften*, 52: 216.

Goncalves, F., Ribeiro, R. and Soares, A. M. V. M., 1995. Laboratory study of effects of temperature and salinity on survival and larval development of a population of *Rhithropanopeus harrisii* from the Mondego River estuary, Portugal. *Mar. Biol.*, 121(4): 639 - 695.

Gopalakrishnan, K., 1976. Larval rearing of red shrimp *Penaeus marginatus* (Crustacea). *Aquaculture*, 9: 145 - 154.

Gopalakrishnan, A., 1991. Studies on some aspects of the reproductive physiology of the female grey mullet *Mugil cephalus* L. Ph. D. Thesis, Cochin University of Science and Technology.

Goswami, S. C. and George, M. J., 1978. Seasonal abundance of penaeid prawn larvae and postlarvae in the coastal waters of Goa up to 50m depth zone. *Ind. J. Mar. Sci.*, 7(2): 110 - 115.

Goswami, S. C. and Goswami, U., 1992. Lunar, diet and tidal variability in penaeid prawn larval abundance in the Mandovi Estuary, Goa. *Ind. J. Mar. Sci.*, 21(1): 21 - 25.

Gunaga, V., Neelakantan, K. and Neelakantan, B., 1989. Seasonal abundance of penaeid postlarvae in the Kali estuary, Karwar. *Mahasagar*, 22(4): 193 - 197.

Gundermann, N. and Popper, D., 1975. Experiment in growing *Penaeus merguiensis* (de Man, 1888) in a fish pond in Fiji. *Aquaculture*, 6: 197 - 198.

* Gunter, G., 1950. Seasonal population changes and distributions as related to salinity of certain invertebrates of the Texas coast, including the commercial shrimp. Publ. Inst. Mar. Sci. Univ. Tex., 1 (2): 7 – 51

Gunter, G., 1961. Some relations of estuarine organisms to salinity. *Limnol. Oceanogr.*, 6(2): 182 - 190.

Gunter, G., Christmas, J. Y. and Killebrew, R., 1964. Some relations of salinity to population distribution of marine organisms, with special reference to penaeid shrimp. *Ecology*, 45: 181 - 185.

Gunter, G., 1967. Some relationships of estuaries to the fiseries of the Gulf of Mexico. *In:* Estuaries, (ed. Lauff, G. H.). American Association Ad. Sci. Publ., Washington D. C., pp. 621 - 638.

Guraya, S. S., 1973. Follicular atresia. Proc. Indian Natn. Sci. Acad., 39B(3): 311 - 332.

* Hagedon, H. H. and Kunkel ,J. G., 1979. Vitellogenin and vitellin in insects - Annual Review of Entomology, 24: 475 - 505.

Halder, D.D., 1978. Induced maturation and breeding of *Penaeus monodon* under brackishwater pond conditions by eyestalk ablation. *Aquaculture*, 15: 171 - 174.

Har, C. H., 1991. Effects of eyestalk ablation, thoracic ganglion extract and gonad extract from 'spent spawners' on ovarian maturation in pond-reared shrimps *Penaeus monodon* Fabricius. *Aquacult. Fish. Manag.*, 22: 463 - 476.

Hara, A., Yamauchi, K. and Hirai, H., 1980. Studies on female specific serum protein (vitellogenin) and egg yolk protein in Japanese eel (*Anguilla japonicus*). *Comp. Biochem. Physiol.*, 65B: 315 - 320.

Hariati, A. M., Wiadnyer, D. G. R., Rini, R. K., Boon, J. H. and Verdegem, M. C. J., 1998. *Penaeus monodon* (Fabricius) and *Penaeus merguiensis* (de Man) biculture in East Java, Indonesia. *Aquacul. Res.*, 29 (10): 743 - 750.

Harpaz, S. and Karplus, I., 1991. Effects of salinity on growth and survival of juvenile *Penaeus semisulcatus* reared in the laboratory. *Israeli J. Aquacul., Bamidgeh*, 43(4): 156 - 163.

Hinsch, G. W. and Bennett, D. C., 1979. Vitellogenesis stimulated by thoracic ganglion implants into destalked immature spider crabs, *Libinia emarginata*. *Tissue and Cell*, 11: 345 - 351.

Horn, E. C. and Kerr, M. S., 1969. The haemolymph proteins of the blue crab *Callinectes sapidus*. I. Haemocyanins and certain other major constituents. *Comp. Biochem. Physiol.*, 29: 193 - 508.

Hossain, M. M., Kader, M. A. and Islam, K. S., 1990. Induced maturation and spawning of reared Indian white prawn *Penaeus indicus* H. Milne Edwards, 1837. *Bangladesh J. Zool.*, 18(2): 245 - 251.

Hossain, M. M., Kader, M. A. and Islam, K. S., 1991. Studies on the sex organs and gonadal maturation in reared Indian white prawn *Penaeus indicus* H. Milne Edwards, 1837. *Bangladesh J. Zool.*, 19(1): 77 - 83.

Hudinaga, M., 1942. Reproduction, development and rearing of *Penaeus japonicus* Bate. *Jap. J Zool.*, 10(2): 305 - 393.

Jasmani, S., Kawazoe, I., Shih, T., Suzuki, Y. and Aida, K., 2000. Haemolymph vitellogenin levels during ovarian development in the kuruma prawn *Penaeus japonicus*. *Fish. Sci.*, 66: 535 - 539.

Jetani, K. L., Savalia, C. S., Patel, P. P., Tank, P. R. and Karena, K. K., 1996. Studies on the maturity and repeated spawning in *Penaeus indicus* in captivity. *Fish. Chimes*, 15(12): 23 - 24.

Joseph, S., 1996. Some studies on the reproduction endocrinology of tiger prawn *Penaeus monodon* Fabricius. Ph. D. Thesis, Cochin University of Science and Technology

Joshi, P. C., Nagabhushanam, R. and Kulkarni, G. K., 1982. Gametogenesis in a marine penaeid prawn *Parapenaeus stylifera* (H. Milne Edwards). *In*: Progress in Invertebrate Reproduction and Aquaculture, Proceedings of the First All India Symposium on Invertebrate Reproduction (ed. Subramoniam, T. and Varadarajan, S.). Indian Society of Invertebrate Reproduction, Madras, pp. 25 - 30.

Junera, H., Zerbib, C., Martin, M. and Meusy, J J., 1977. Evidence for control of vitellogenin synthesis by an ovarian hormone in *Orchestia gammarella* (Pallas). Crustacea, Amphipoda. *Gen. Comp. Endocrinol.*, 31: 457 - 462.

Kanazawa, A. and Teshima, S., 1971. In vivo conversion of cholesterol to steroid hormones in the spiny lobster, *Panulirus japonicus*. *Bull. Jpn. Soc. Sci. Fish.*, 37: 891 - 898.

Kawahigashi, D., 1992. A survey of commercial maturation technology in the western hemisphere. In: Proceedings of Special Session on Shrimp Farming (ed. Wyban, J.). World Aquaculture Soceity, Baton, Rogue, USA, pp. 52 - 54.

Kawazoe, I., Jasmani, S., Shih, T., Suzuki, Y. and Aida, K., 2000. Purification and characterization of vitellin from the ovary of kuruma prawn *Penaeus japonicus*. *Fish. Sci.*, 66: 390 - 396.

Kelemec, J. A. and Smith, I. R., 1980. Induced ovarian development and spawning of *Penaeus plebejus* in a recirculating laboratory tank after unilateral ablation. *Aquaculture*, 21(1): 55 - 62.

Kennedy, F. S., Crane, J. J., Schlieder, R. A. and Barber, D. G., 1977. Studies of the rock shrimp *Sicyonia brevirostris*, a new fishery resource on Florida's Atlantic shelf. Fla. Mar. Res. Publ. No.27, pp. 215.

Khayat, M., Lubzen, E., Tietz, A. and Funkenstein, B., 1994. Cell-free synthesis of vitellin in the shrimp *Penaeus semisulcatus* (de Haan). *Gen. Comp. Endocrinol.*, 93: 205 - 213.

King, J. E., 1948. A study of the reproductive organs of the common marine shrimp *Penaus setiferus* (L). *Biol. Bull.*, 94: 244 - 262.

Kinne, O., 1970a. Temperature 3.3 Animals, 3.31 Invertebrates. *In:* Marine Ecology, 1(1). (ed. Kinne, O.). Wiley - Interscience, London, New York, Sydney, Toronto, pp. 407 - 514.

Kinne, O., 1970b. Salinity 4.3 Animals, 4.31 Invertebrates. *In:* Marine Ecology, 1(2). (ed. Kinne, O.). Wiley - Interscience, London, New York, Sydney, Toronto, pp. 821 -995

Komm, B. S. and Hinsch, G. W., 1987a. Oogenesis in the terrestrial hermit crab, *Coenabita clypeatus*. 1. Previtellogenic oocytes. *J. Morphol.*, 183: 219 - 224.

Komm, B. S. and Hinsch, G. W., 1987b. Oogenesis in the terrestrial hermit crab, *Coenabita clypeatus*. 1. Vitellogenesis. *J. Morphol.*, 192: 269- 277.

Kulkarni, G. K., Nagabhushanam, R. and Joshi, P. K., 1979. Effect of progesterone on ovarian maturation in a marine penaeid prawn *Parapenaeopsis hardwickii* (Miers 1878). *Indian J. Exp. Biol.*, 17: 986-987.

Kulkarni, G. K. and Nagabhushanam, R., 1980. Role of ovary-inhibiting hormone from eyestalks of marine penaeid prawns (*Parapenaeopsis hardwickii*) during ovarian development cycle. *Aquaculture*, 19: 13- 19.

Kulkarni, G. K., Nagabhushanam, R. and Joshi, P. K., 1981 . Neuroendocrine regulation in the marine penaeid prawn, *Parapenaeopsis hardwickii. Indian J. Mar. Sci.*, 10(4): 350- 352.

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: 513-519.

Kulkarni, G. K. and Fingerman, M., 1992. Effects of 5-hydrxytryptamine agonists on ovarian development in the fiddler crab, *Uca pugilator. Comp. Biochem. Physiol.*, 101C: 419- 423.

Kulkarni, G. K., Nagabhushanam , R., Amaldoss, G., Jaiswal, R. G. and Fingerman, M., 1992. In vivo stimulation of ovarian development in the red swamp crayfish, *Procambarus clarkii* (Girard), by 5 - hydroxytryptamine. *Invert. Rep. Dev.*, 21: 231- 240.

Kumulu, M. and Jones, D. A., 1995. Salinity tolerance of hatchery-reared postlarvae of *Penaeus indicus* H. Milne Edwards originating from India. *Aquaculture*, 130: 287- 296.

Kumulu, M., Eroldogan, O. T. and Aktas, M., 1999. The effect of salinity on larval growth, survival and development of *Penaeus semisulcatus* (Decapoda: Penaeidae). *Israeli J. Aquaculture- Bamidgeh*, 51(3): 114 –121.

* Kutkuhn, J. H., 1966. The role of estuaries in the development and perpetuation of commercial shrimp resources. *Am. Fish. Soc. Spec. Pub.*, 3: 16- 36.

Kuttyamma, K. J., 1982. The effect of salinity on the growth of some penaeid prawn. *Bull. Dept. Mar. Sci. Univ. Cochin.*, 8: 1- 34.

Laemmli, U. K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680- 685.

Lakshmikantham, K. P., 1982. Salinity tolerance of postlarvae of *Penaeus indicus* H. Milne Edwards. M. Sc. Dissertation, Univ. Cochin, pp. 77.

Lam, T. J., Pandey, S., Nagahama, Y.and Hoar, W. S., 1978. Endocrine control of oogenesis, ovulation and oviposition in goldfish. *In:* Comparative Endocrinology (ed. Gaillard, P. J. and Boer, H. H.). Elsevier, Amsterdam, pp. 55 - 64

Laufer, H., Borst, D., Baker, F. C., Carrasco, C., Sinkus, M., Reuter, C. C., Tsai, L. W. and Schooley, D. A., 1987. Identification of a juvenile hormone-like compound in a crustacean. *Science*, 235: 202 - 205.

Laufer, H. and Downer, R. G. H., 1988. Endocrinology of selected Invertebrate types. Alan, R. L. Inc., New York, pp. 500.

Lawrence, A. L., Akamine, Y., Middleditch, B. S., Chamberlain, G. W. and Hutchins, D., 1980. Maturation and reproduction of *Penaeus setiferus* in captivity. Proc. World Maricul. Soc., 11: 481 - 487.

Laxmilatha, P., 1991. Studies on the haemolymph of *Penaeus indicus* H. Milne Edwards. Ph. D. Thesis, Cochin University of Science and Technology

Lee, F., Shiha, T. and Cheng, C., 1997. Isolation and characterization of the female-specific protein (vitellogenin) in mature female haemolymph of the freshwater prawn *Macrobrachium rosenbergii*: Comparison with ovarian vitellin. *Gen. Comp. Endocrionol.*, 108: 406 - 415.

Lester, C. J., 1984. Genetic changes during development of penaeid shrimp. *In*: Proceedings of the first International Conference on the culture of Penaeid shrimps/ Prawns. (ed. Taki, Y., Primavera, J. H. and Lloberra, J. A.). SEAFDEC, Iloilo, pp. 173.

Lester, L. J. and Pante, J. R., 1991. Penaeid temperature and salinity responses. *In* : Marine shrimp culture: Principles and Practices (ed. Fast, A. W. and Lester, L. J.). Elsevier, Amsterdam, pp. 515 - 534.

Lim, L. C., Heng, H. H. and Cheong, L., 1987. Manual on breeding of banana prawn. Primary Production Department, Ministry of National Development. Republic of Singapore. Fisheries Handbook No. 3: 1 - 62.

Linder, M. J. and Cook , H. L., 1970. Synopsis of biological data on white shrimp, *Penaeus setiferus* Linnaeus. *Biol. Bull.*, 94 (3): 224 - 262.

Longyant, S., Sithigorngul, P., Thampalerd, N., Sithigorngul, W. and Menasveta, P., 1999. Monoclonal antibodies production specific to vitellin and vitellogenin of giant tiger prawn *Penaeus monodon*. *Invert. Reprod. Dev.*, 35: 9 - 17.

Longyant, S., Sithigorngul, P., Thampalerd, N., Sithigorngul, W. and Menasveta, P., 2000. Characterization of vitellin and vitellogenin of giant tiger prawn *Penaeus monodon* using monoclonal antibodies specific to vitellin subunits. *Invert. Reprod. Dev.*, 37 (3): 211 - 221.

Lovett, D. L. and Felder, D. L., 1998. Evaluation of rotifer *Brachionus plicatilis* as a substitute for *Artemia* in feeding larvae of *Macrobrachium rosenbergii*. *Aquaculture*, 71: 331 - 338.

Lubzens, E., Ravid, T., Khayat, M., Daube, N. and Tietz, A., 1997. Isolation and characterization of lipoprotein from the haemolymph and ovary of penaeid shrimp *Penaeus semisulcatus* (de Haan): Apoprotein and lipids. *J. Exp. Zool.*, 278: 339 - 348.

Lui ,C. W., Sage. A. and O'Connor, J. D., 1974. Biosynthesis of lipovitellin by the crustacean ovary. *J. Exp. Zool.*, 188: 289 - 296.

Lui, C. W. and O'Connor, J. D, 1976. Biosynthesis by the crustacean ovary. II. Characterization of and in vitro incorporation of amino acids into the purified subunits. *J. Exp. Zool.*, 195: 41 - 52.

Lui, C. W. and O'Connor, J. D., 1977. Biosynthesis of crustacean lipovitellin. III. The incorporation of labelled aminoacids into the purified subunits. *J. Exp. Zool.*, 199: 105 - 108.

Lui, H. and Lui, R., 1994. Comparative study on postlarval development of penaeid shrimps, *Penaeus chinensis, P. penicilatus* and *P. merguiensis*. Stud. Mar. Sin./ Haiyabg Kexue Jikam, 35: 179 - 238.

Lumare, F., 1979. Reproduction of *Penaeus kerathurus* using eyestalk ablation. *Aquaculture*, 18: 203 - 214.

Lumare, F., Di- Muro, P., Tenderini, L. and Zupo, V., 1993. Experimental intensive culture of *Penaeus monodon* in a fishery "valle" of the river Po Delta, on the north-east coast of Italy. *Aquaculture*, 13: 231 - 241.

Lumare, F., Scordella, G., Zanella, L. and Tessarin, C., 1996. Ovarian maturity and reproduction in captivity of *Penaeus monodon* (Decapoda, Penaeidae) on the north-east coast of Italy. *Obelia*, 22: 65 - 70.

Lynn, J. W., Pillai, M. C., Glas, P. S and Green, J. D, 1991. Comparative morphology and physiology of egg activation in selected Penaeoidea. *In:* Frontiers in Shrimp Research (ed. De Loach, P.F., Dougherty, W. J. and Davidson, M. A.). Elsevier, Amsterdam. 47 - 64.

MacKenzie, B. R., 1985. Assessment of temperature effects on inter-relationship between stage durations, mortality and growth in laboratory reared *Homarus americanus* Milne Edwards larvae. *J. Exp. Mar. Biol. Ecol.*, 116(1): 87 - 98.

Mair, J. McD., 1980. Salinity and water-type preference of four species of postlarval shrimp (Penaeus) from west Mexico. *J. Exp. Mar. Biol. Ecol.*, 183: 133 - 145.

Makinouchi, S. and Primavera, J. H., 1987. Maturation and spawning of *Penaeus indicus* using different ablation methods. *Aquaculture,* 62(1) : 73 - 81.

Makinouchi, S. and Hirata, H., 1995 . Studies on maturation and reproduction of pond reared *Penaeus monodon* for developing a closed life cycle culture system. *Israeli J. Aquacult., Bamidgeh*, 47(2): 68 - 77.

Marian, M. P. and Murugadass, S., 1991. Effect of eyestalk ablation on egg production and food conversion efficiency of the commercial riverine prawn *Macrobrachium malcolmsonii. In:* Larvi '91 (ed. Lavens, P., Sorgellos, P., Jaspers, E. and Ollevier, F.). Spec. Publ. Eur. Aquacult. Soc.,15: 260 - 263.

Marzari , R., Ferrero, E., Moses, A. and Savoini, A., 1986. Immunological characterisation of vitellogenic proteins in Squilla mantis haemolymph, Crustacea, Stomatopoda. *Exp. Biol.*, 45: 75 - 80

Matsuyama, M. and Matsuura, S., 1983. On the maturation of the korai prawn, *Penaeus chinensis* (Osbeck) in the Yellow Sea. *Sci. Bull. Fac. Agr. Kyushu Univ.*, 38: 71 - 79.

McTigue, T. A. and Zimmerman, R. J., 1991. Carnivory versus herbivory in juvenile *Penaeus setiferus* (Linnaeus) and *P. aztecus* (Ives). *J. Exp. Mar. Biol. Ecol.*, 15: 1 - 6.

Medidna, A., Vila, Y., Mourente, G and Rodriguez, A., 1996. A comparative study of the ovarian development in wild and pond reared shrimp *Penaeus kerathurus* (Forskal 1775). *Aquaculture*, 148: 63 - 75.

Menasveta, P., Piyatiratitivorakul, S., Rungsupe, S., Moore, N. and Fast, A. W., 1993. Gonadal maturation and reproductive performance of giant tiger prawn (*Penaeus monodon* Fabricius) from the Andaman sea and pond reared sources in Thailand. *Aquaculture*, 116: 191 - 198.

Menasveta, P., Sangpradub, S., Piyatiratitivorakul S., Vorakul ,S. and Fast ,A. W., 1994. Effect of broodstock size and source on ovarian maturation and spawning of *Penaeus monodon* Fabricius from the Gulf of Thailand. *J. World Aquacul. Soc.*, 25: 41 - 49.

Meusy, J. J., 1980. Vitellogenin, the extraovarian precursor of the protein yolk in Crustacea: a review. *Rep. Nutrition Develop.*, 20: 1 - 21.

Meusy, J. J., Junera, H., Cledon, P. and Marlin, M., 1983. La vitellogenine chez un Crustace` Decapode Natantia, *Palaemon serratus* Mise en evidence, comparison immunologique avec les vitellines, site de synthese et role des pedoncules oculaires. *Rep. Nutrition Develop.*, 23: 625 - 640.

Meusy, J. J. and Payen, G. P., 1988. Female reproduction in malacostracan Crustacea. *Zool. Sci.*, 5: 217 - 265.

Minagawa, M., Chiu, J. R., Kudo, M., Ito, F. and Takashima. F., 1993. Female reproduction biology and oocyte development of the red frog crab, *Ranina ranina*, off Hachijojima, Izu Islands, Japan. *Mar. Biol.*, 115: 613 - 623.

Minagawa, M. and Sano, M., 1997. Oogenesis and ovarian development cycle of the spiny lobster *Panulirus japoncus* (Decapoda: Palinuridae). *Mar. Freshwater Res.*, 48: 875 - 887.

Mohamed, K. H., Muthu, M. S., Pillai, N. N. and George, K. V., 1978. Larval development of *Metapenaeus monoceros* (Fabricius). Bull. Cent. Mar. Fish Res. Inst., 28: 50 -59.

Mohamed, K. S., 1989. Studies on the reproductive ndocrinology of the penaeid prawn *Penaeus indicus* H. Milne Edwards, Ph. D. Thesis, Cochin University of Science and Technology, pp. 250.

Mohamed, K. S. and Diwan, A. D., 1994. Vitellogenesis in the Indian white prawn *Penaeus indicus* (Crustacea: Decapoda: Penaeidae). *J. Aquacul. Trop.*, 9: 157 - 172.

Montenegro, H. and Moreira , G., 1992. Temperature and salinity effects on respiratory metabolism of selected embryonic stages of *Macrobrachium olfersii* (Wiegmann) (Decapoda: Palaemonidae). *In*: First European Crustacean Conference. Paris, (Abstract), pp. 102 -103.

Motoh, H., 1978. Preliminary histological study of ovarian development of the giant tiger prawn *Penaeus monodon*. *Quart. Res. Rep.*, 2: 4 - 6.

Motoh, H. and Buri, P., 1979. Larvae of decapod crustacea of the Philippines-IV. Larval development of the banana prawn, *Penaeus merguiensis* reared in the laboratory. *Bull. Jap. Soc. Sci. Fish.*, 45: 1217 - 1235.

Munro, I. S. R., 1975. Biology of the banana prawn (*Penaeus merguiensis)* in the southeast corner of the Gulf of Carpentaria. *In:* Second Australian National Prawn Seminar (ed. Younge, P. C.).NPSZ, Cleveland, Australia, pp. 60 - 78.

Munuswamy, N. and Subramoniam, T., 1987. Sexual and developmental differences in the protein pattern of haemolymph and body tissues of *Streptocephalus dichotomus* Baird (Crustacea: Anostraca). *Hydrobiologia,* 150: 177 - 183.

Murugesan, P., Rajagopal, S. and Khan, S. A., 1998. Utility of eyestalk ablation in farming and broodstock development of the shrimp *Penaeus monodon* Fabricius. *Indian J. Exp. Biol.,* 36(7): 699 - 702.

Muthu, M. S., Pillai, N. N. and George, K. V., 1978a. Larval development – *Metapenaeus dobsoni.* In: Larval development of Indian penaeid prawn. Bull. Cent. Mar. Fish Res. Inst., 28: 30 - 40.

Muthu, M. S., Pillai, N. N. and George, K. V., 1978b. Larval development-*Metapenaeus affinis.* In: Larval development of Indian penaeid prawn. Bull. Cent. Mar. Fish Res. Inst., 28: 40 - 50.

Muthu, M. S. and Laxminarayana, A., 1982. Induced maturation of penaeid prawn-a review. Proc. Symp. Coastal Aquacul., 1: 16 - 27.

Nagabhushanam, R. and Kulkarni, G. K., 1982. Reproductive biology of the female of a penaeid prawn *Parapenaeopsis hardwickii* (Miers). *Indian J. Fish.,* 29 (1& 2): 151 - 159.

Nagabhushanam, R. and Joshi, P. K., 1986. Effect of environmental factors on the neurosecretory cells in *Parapenaeopsis stylifera.* Proc. Indian Natl. Acad. Sci., 56(2): 89 - 93.

Nagabhushanam, R., Sarojini, R. and Sambasiva Rao, S., 1992. Reproductive endocrinology of the Indian marine commercial shrimp *Metapenaeus affinis. In:* Aquaculture research needs for 2000A. D, pp. 163 - 179.

Nair, S., Sreekumaran, R. and Krishnankutty, M., 1975. Note on the varying effects of salinity on the growth of the juveniles of *Penaeus indicus* from Cochin backwaters. Bull. Dept. Mar. Sci. Univ.Cochin, 8(1): 181 - 184.

Nandakumar, G., Pillai, N. N., Telang, K. Y. and Balachandran, K., 1989. Larval development of *Macrobrachium moyebi* (Kishinouye) in the laboratory. *J. Mar. Biol. Assoc.*, 31(1-2): 86 - 102.

Navas, K. A. and. Sebastian, M. J, 1989. Effect of low salinities on the survival and growth of *Penaeus monodon* (Fabricius). *Indian J. Fish.*, 36(3): 257 - 261.

Nelson, K., Heyer, B., Johnson, E., Hedgecock, D. and Chang, E. S., 1988. Photoperiod-induced changes in haemolymph in female lobsters (*Homarus americanus*). *Comp. Biochem. Physiol.*, 90B: 809 - 821.

O'Brien, C. J., 1994. The effects of temperature and salinity on growth and survival of juvenile tiger prawns *Penaeus esculentus* (Haswell). *J. Exp. Mar. Biol. Ecol.*, 133 - 145.

O'Donovan, P., Abraham, M. and Cohe, D., 1984. The ovarian cycle during the intermoult in ovigerous *Macrobrachium rosenbergii*. *Aquaculture*, 36: 347 - 358.

Oguri, M. and Takada, N., 1967. Serum calcium and magnesium levels of gold fish with special reference to their gonadal maturation. *Bull. Jap. Soc. Sci. Fish*, 33(3): 161 - 166.

Oka, M. and Shirahata ,S., 1965. Studies on *Penaeus orientalis* Kishinouye-II. Morphological classification of the ovarian stages and the maturity of the ovary. *Bull. Fac. Fish. Nagasaki Univ.*, 18: 30 - 40. (in Japanese with English abstracts)

Oka, M., 1967. Studies of *Penaeus orientalis* Kishinouye .III - structure of ovary and mechanism of ovulation. *Bull. Fac. Fish. Nagasaki Univ.*, 23: 46-56 (in Japanese with English abstracts)

Okumaura, T. and Aida, K., 2000. Haemolymph vitellogenin levels and ovarian development during the reproductive and non- reproductive moult cycles in the giant freshwater prawn *Macrobrachium rosenbergii*. *Fish. Sci.*, 66: 678 - 685.

Oliviera, P. S. P. and Correa, A. M. A., 1999. Effect of 5-HT and eyestalk monoablation on the induction of ovarian maturation in *Penaeus penicillatus*. *Rev. Bras. Biol.*, 59(2): 351 - 358.

Omori, M., 1979. Growth, feeding and mortality of larval and early postlarval stage of the oceanic shrimp *Sergestes simitis* (Hansen). *Limnol. Oceanogr.*, 24: 273 - 288.

Oogle, J. T., Beaugez, K. A. and Mc Ilwain, T. D., 1988. Effect of salinity on growth of *Penaeus vannamei* postlarvae. (Abstract). *J. World Aquacul. Soc.*, 19(1): 54A

* Otsu, J. and Hanoaka, K. I., 1951. Relation between body weight and precocious differentiation of ova in eyestalkless crabs. *Bull. Yamagata Univ.* (Nat. Sci.), 1: 269 - 274.

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

* Oyama, S. N., 1968. Neuroendocrine effects on ovarian development in the crab *Thalamitta crenata* Latreille studied in vitro. Doctoral dissertation. University of Hawaii, Honolulu, Hawaii, USA.

Palacios, E., Rodriguez-Jaramillo ,C. and Racotta, I. S.,1999. Comparison of the ovary histology between different - sized wild and pond reared shrimp *Litopenaeus vannamei* (= *Penaeus vannamei*). *Invert. Reprod. Dev.*, 35(3): 251 - 259.

Panouse, J., 1943. Influence de l'ablation du pedoncle oculaire sur la croissance de l'ovarre chez la crevette *Leander serratus*. C. R. Acad. Sci., 217: 553 - 555.

Pantastico, J. B., 1979. Research paper presented at the "Technical Consultation on Available Aquaculture Technology in the Philippines". South-East Asian Fisheries Development Centre, Aquaculture Department, Tigbauan, Iloilo, Philippines, pp. 5.

Papathanassiou, E. and King, P. E., 1984. Ultrastructural studies on gametogenesis of the prawn *Palaemon serratus* (Pennant) 1. Oogenesis. *Acta. Zool.*, 65(1): 17 - 31.

Parado - Estepe, F. D., Llobrero, J. A., Villaluz, A. and Salde, R., 1993. Survival and metamorphosis of *Penaeus monodon* larvae at different salinity levels. *Israeli J. Aquacult., Bamidgeh*, 45(1): 3 - 7.

Parado - Estepa, F. D., 1998. Survival of *Penaeus monodon* postlarvae and juveniles at different salinity and temperature levels. *Israeli J. Aquacult., Bamidgeh*, 50(4): 174 - 183.

Parulekar, A. H., Achuthankutty C. T., 1993. Resource potential of juvenile marine prawns in the estuaries of Goa. Tech. Rep. No. NIO/ SP-2/93, pp. 85.

Passano, L. M., 1960. Moulting and its control. *In:* Physiology of Crustacea. Vol. 1, (ed. Waterman, T. H.), Academic Press, New York, pp. 473 - 536.

Paula, J., Costlow, J. D., Scelzo, M. A. and Monroe, K., 1992. Influence of temperature and salinity on the larval development of estuarine decapod crustaceans, *Sesarama reticulatum*. *In:* First European Crust. Conference. Paris. (Abstract), pp. 4.

Paulus, J. E. and Laufer, H., 1982 . Vitellogenesis in the hepatopancreas and ovaries of *Carcinus maenas*. *Biol. Bull.*, 163: 355 - 404.

Paulus, J. E. and Laufer, H., 1987. Viteliogenocytes in hepatopancreas of Carcinus maenas and *Libinia emarginata* (Decapoda, Branchyura). *Int. J. Invert. Reprod. Dev.*, 11: 29 - 44.

Payen, G. G., 1986. Endocrine regulation of male and female genital activity: a retrospect and perspectives. *In:* Advances in Invertebrates Reproduction , Vol. 4 (ed. Porchet, M., Andries, J. C. and Dhainaut, A.), Elsevier, Amsterdam, pp. 125 - 134.

Penn, J. W., 1980. Spawning and fecundity of the westernking prawn *Penaeus latisulcatus* Kishinouye, in western Australian waters. *Aust. J. Mar. Freshwat. Res.*, 31: 21- 35.

Peterson, G. L. and Shehadeh, Z. H., 1971. Changes in blood components of the mullet, *Mugil cephalus* L., following treatment with salmon gonadotropin and methyl testosterone. *Comp. Biochem. Physiol.*, 78B: 451 - 457.

Picaud, J. L., 1980. Vitellogenin synthesis by the fat body of *Porcellio dilatatus* Brandt (Crustacea, Isopoda). *Invert. Reprod. Dev.*, 2: 341 - 349.

Picaud, J. L., Souty, C. G and Martin, G., 1989. Vitellogenesis in a dacapod crustacean *Palaemon serratus*, identification, immunological similarity to vitellin synthesis and role of eyestalk. *Reprod. Nutr Dev.*, 23: 625 - 640.

Pillai, R. S. and Nair, N. B., 1970. Studies on the shrimp *Cardina laevis* (Heller) II. The reproductive system. *J. Mar. Biol. Assoc., India*, 2: 226 - 236.

Pillay, K. K. and Nair, N. B., 1971. The annual reproduction cycles of *Uca annulipes*, *Portunus pelagicus* and *Metapenaeus affinis* (Decapoda: Crustacea) from south-west coast of India. *Mar. Biol.*, 11: 152 - 166.

Pillai, N. N., 1991. Studies on the life history of some carrideans of the southwest coast of India. Ph. D. Thesis, Cochin University of Science and Technology

Ponce - Palafox, J., Martinez - Palacios, C. A. and Ross, L. G., 1997. The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp *Penaeus vannamei* Boone, 1931. *Aquaculture*, 157: 107 - 115.

Prasad, C. V. N., Prasad, P. N. and Neelakantan, B., 1988. Salinity tolerance in the larvae (mysis III) of banana prawn *Penaeus merguiensis* (De Man). *Seafood Export Journal*, 20 (9): 21 - 26.

Preston, N., 1985. The combined effects of temperature and salinity on hatching success and the survival, frowth and development of the larval stages of *Metapenaeus bennettae* (Racek & Dall). *J. Exp. Mar. Biol. Ecol.*, 85: 57 - 74.

Primavera, J. H., 1978. Induced maturation and spawning in five month old *Penaeus monodon* Fabricius by eyestalk ablation. *Aquaculture*, 13: 355 - 359.

Primavera, J. H. and Posadas, R. A., 1981. Studies on the egg quality of *Penaeus monodon* Fabricius based on morphology and hatching rate. *Aquaculture*, 22(3): 269 - 277.

Primavera, J. H., 1982. Broodstock of sugpo, *Penaeus monodon* Fabricius. Extension Manual No.7, 3rd edn., Aquaculture Dept., Southeast Asian Fisheries Development Centre, Iloilo, Philippines, pp. 26.

Primavera, J. H., 1985. A review of maturation and reproduction in closed thelycun penaeids. *In*: Proceedings of the first International Conference on the culture of Penaeid shrimps/ Prawns. (ed. Taki, Y., Primavera, J. H. and Lloberra, J. A.). SEAFDEC, Iloilo, pp. 47 - 64.

Primavera, J. H. and Caballero, R.M.H., 1992. Light colour and ovarian maturation in unablated and ablated giant tiger prawn *Penaeus monodon*. *Aquaculture*, 108: 247 - 256.

Pudadera, R. A. and Primavera, J. H., 1981. Effect of light quality and eyestalk ablation on ovarian maturation in *Penaeus monodon*. Kalikasan, 10(2/3): 231 - 241.

Qiu, Y. W., T. B. Ng. and Chu, K. H., 1997. Purification and characterization of vitellin from the ovaries of the shrimp *Metapenaeus ensis* (Crustacea: Decapoda: Penaeidae). *Int. J. Invert. Reprod. Dev.*, 31: 217 - 223.

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

Quackenbush, L. S., 1986. Crustacean endocrinology, a review. *Can. J. Fish. Aq. Sci.*, 43: 2271 - 2282.

Quackenbush, L. S. and Keeley, L. L., 1988. Regulation of vitellogenesis in the fiddler crab *Uca pugilator. Biol. Bull.*, 175: 321 - 331.

Quackenbush, L. S., 1989a. Vitellogenesis in the shrimp, *Penaeus vannamei*, in vitro studies of the isolated hepatopancreas and ovary. *Comp. Biochem. Physiol.*, 94B: 253 - 261.

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

Quackenbush, L. S., 1991. Regulation of vitellogenesis in penaeid shrimp. *In:* Frontiers in Shrimp Research (ed. De Loach, P. Dougherty, W. J., Davidson, M. A.). Elsevier, Amsterdam, pp. 125 - 140.

Quinitio, E. T. and Villegas, C. T., 1982. Growth, survival amd macronutrient composition of *Penaeus monodon* Fabricius larvae fed with *Chaetoceros calcitrans* and *Tetraselmis chuii. Aquaculture,* 29: 253 - 260.

Quinitio, E. T., Hara, A., Yamauchi, K., Mizushima, T. and Fuji, A., 1989. Identification and characteization of vitellin in a hermaphrodite shrimp, *Pandalus kessleri* . *Comp. Biochem. Physiol.*, 94B: 445 - 451.

Quinitio, E. T., Hara, A., Yamauchi, K. and Fuji, A., 1990. Isolation and characterization of vitellin from the ovary of *Penaeus monodon. Int. J. invert. Reprod. Dev.*, 17: 221 - 227.

Quinitio, E. T. and Millamena, O. M., 1992. Ovarian changes and female specific protein levels during sexual maturation of the white shrimp *Penaeus indicus. Israeli J. Aquacult., Bamidgeh,* 44: 7 - 12.

Quinitio, E.T., Caballero, R. M. and Gustilo, L., 1993. Ovarian development in relation to changes in the external genitalia in captive *Penaeus monodon. Aquaculture*, 114: 71 - 81.

Ragunathan, M. G. and Arivazhagan, A., 1999. Influence of eyestalk ablation and 5-hydroxytryptamine on the gondal development of a female crab, *Paratelphusa hydrodromous* (Herbst). *Curr. Sci.*, 76(4): 583 - 587.

Rahaman, A. A., 1967. Reproductive and nutritional cycle of the crab *Portunus pelagicus* of Madras Coast. Proc. Indian Acad. Sci., 65: 76 - 82.

Raj, P. R. and Raj ,P. J . S., 1982. Effect of salinity on growth and survival of three species of penaeids. Proc. Symp. Coastal Aquacult., 12 - 18, 1: 31 - 35.

Raje, C. P. and Ranade, M. R., 1972. Larval developments of Indian penaeid shrimps - 1. *Penaeus merguiensis de Man. J. Indian Fish. Assoc.*, 2: 1 - 16.

Rajyalakshmi, T. and Chandra, D. M., 1987. On culture of *Penaeus monodon* in saline and freshwater ponds in Andra Pradesh, India. *Indian J. Anim. Sci.*, 57: 348 - 358.

Ramos, L., Espejo, M., Samada, S.and Perez, L., 1995. Maturation and reproduction of pond reared *Penaeus schmitti. J. World Aquacul. Soc.*, 26(2): 183 - 187.

Rangnekar, P. V. and Deshmukh, R. D., 1968. Effect of eyestalk removal on the ovarian growth of the marine crab, *Scylla serrata. J. Anim. Morphol. Physiol.*, 15: 116 - 126.

Rankin, S. M., Bradfield, J. Y. and Keeley, L. L., 1989. Ovarian protein synthesis in the South American white shrimp. *Penaeus vannamei*, during the reproductive cycle. *Invert. Reprod. Dev.*, 15: 27 - 33.

Rao, P. V., 1967. Maturation and spawning of the penaeid prawns of the southwest coast of India. FAO Fish. Rep., 57: 285 - 302.

Rao, P. V., 1973a. Studies on the larval development of the commercially important penaeid prawns of India. *J. Mar. Biol. Assoc.*, 15(1): 95 - 124.

Rao, P. V., 1973b. Some observations on the larval growth of the commercially important penaeid prawns of Southwest coast of India. *Indian J. Fish.*, 20 (2): 308 - 317.

Rasmussen, T. and Tende, K., 1995. Temperature dependent development, growth and mortality in larvae of the deep-water prawn *Pandalus borealis* reared in the laboratory. *Mar. Ecol. Prog. Ser.*, 118 (1-3): 149 - 157.

Reddy, S. and Ramamurthi, P., 1998. Methyl farnesoate stimulates ovarian maturation in the freshwater crab *Oziotelphusa senex* senex. *Curr. Sci.*, 74(1): 68 - 70.

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.*, 106C: 321 - 325.

Roberts, M., 1971. Larval development of *Pagurus longicarpus* (Say) reared in the laboratory. II. Effects of reduced salinity on larval development. *Biol. Bull.*, 140: 104 - 116.

Robertson, L., Bray, B., Samocha, T. and Lawrence, A., 1993. Reproduction of penaeid shrimp: an operations guide. In: CRC Handbook of Mariculture Vol. 1: Crustacean Aquaculture (ed. McVey, J. P.), CRC Press, Boca Raton, FL, USA, pp. 107 - 132.

Ronquillo, J. D. and Saisho, T., 1993. Early developmental stages of greasy back shrimp, *Metapenaeus ensis* (de Haan, 1844) (Crustacea, Decapoda, Penaeidae). *J. Plankton Res*, 15(10): 1177 - 1206.

Rosas, C. L. Ocampo, Gaxiola, G., Sanchez, A. and Soto, L. A., 1999. Effect of salinity on survival, growth and oxygen consumption of postlarvae (PL10 - PL21) of *Litopenaeus setiferus*. *J. Crust. Biol.*, 19(2): 244 - 251.

Rosenberry, R., 1997. World Shrimp Farming' 97. Annual Reports. Shrimp News International, pp. 21.

Rothlisberg, P.C., Crocos. P. J. and Smith, D. M., 1991. The effect of diet and eyestalk ablation on maturation, spawning hatching, and larval fitness of *Penaeus esculentus*. *In:* LARVI' 91. (ed. Lavens, P., Sorgeloos, P., Jaspers, E.; Ollevier, F.). Spec. Publ. Eur. Aquacult. Soc.,15: 247 - 250.

Ruangpanit, N., Maneewongsa, S., Tattanon, T. and Kraisingdeja, P., 1984. Study on the larval rearing of *Penaeus merguiensis*. *In*: Proceedings of the first International Conference on the culture of Penaeid shrimps/ Prawns. (ed. Taki, Y., Primavera, J. H. and Lloberra, J. A.). SEAFDEC, Iloilo, pp. 65 - 78.

Sadhana, M. and Neelakantan, B., 1996. Effect of different protein levels in the purified diets on growth of *Penaeus merguiensis* De Man and feed conversion ratio. *Indian J. Fish.*, 43(1): 61 - 67.

Sadhana, M. and Neelakantan, B., 1997. Growth response of juvenile shrimp *Penaeus merguiensis* (Eucarida/ Crustacea) to feed containing different protein sources. *Indian J. Mar. Sci.*, 26(2): 180 - 185.

Sagi, A. and Ra'Anan, Z., 1985. Rapid identification of reproductive state and the receptive period of females in pond populations of *Macrobrachium rosenbergii* - a new technique. *Aquaculture*, 48: 361 - 367.

Sagi, A., Soroka, Y., Snir, E., Chomsy, O., Calderon, J. and Miner ,Y., 1995. Ovarian protein synthesis in the prawn, *Macrobrachium rosenbergii*. Does ovarian vitellin synthesis exist. *Invert. Reprod. Dev.*, 27(1): 41 - 47.

Sakaji, H., Tsuchiya, K. and Segawa, S., 2000. Oocyte development of *Metapenaeopsis dalei* (Penaeidae, Decapoda, Crustacea). *Invert. Reprod. Dev.*, 38(1): 7 -12.

Samocha, T. M., Lawrence, A. L. and Pooser, D., 1998 . Growth and survival of juvenile *Penaeus vannamei* in low saline waters in a semi-closed recirculatory system. *Israeli J. Aquacul .. Bamidgeh*, 50(2): 55 - 59.

Sandoval Quintero, M. E. and Gracia , A., 1998. Stages of gonadal development in the spotted shrimps *Penaeus brasiliensis*. *J. Crust. Biol.*, 18(4): 680 - 685.

Santiago, A. C. Jr., 1977. Successful spawning of cultured *Penaeus monodon* Fabricius after eyestalk ablation. *Aquaculture*, 11: 185 - 196.

Sarojini, R., Mirajkar, M. S. and Nagabhushanam, K., 1985. Effect of steroids on ovarian development in freshwater prawn, *Macrobrachium kistnensis*. *Comp. Phy. Ecol.*, 10. 7 -11.

Sarojini, R., Jayalakshmi, K. and Sambashvarao, S., 1986. Effect of external steroids on oogenesis and spermatogenesis of the freshwater prawn *Macrobrachium lamerii*. *J. Adv. Zool.*, 7: 50 - 53.

Sarojini, R., Nagabhushanam, R. and Fingerman, M., 1995. Mode of action of the neurotransmitter 5-hydroxytrptamine in stimulating ovarian maturation in the red swamp crayfish, *Procambarus clarkii*: An *in vivo* and *in vitro* study. *J. Exp. Zool.*, 271: 395 - 400.

Sedgewick, R. W., 1979. Influence of dietary protein and energy on growth, food consumption and food conversion efficiency in *Penaeus merguiensis* de Man. *Aquaculture*, 16: 7 - 30.

Sedgewick, R. W., 1980. The requirements of *Penaeus merguiensis* for vitamins and mineral supplements in diets based on freeze-dried *Mytillus edulis* meal. *Aquaculture*, 19: 127 - 137.

Selvakumar, R. A., George, M. J., Achuthankutty, C. T. and Goswami, S. C., 1977. Penaeid prawn larval abundance in the Mandovi estuary, Goa. *Indian J. Mar. Sci.*, 6: 160 - 162.

Shafir, S., Tom, M., Ovadia, M. and Lubzens, E., 1992. Protein, vitellogenin and vitellin levels in the haemolymph and ovaries during ovarian development in *Penaeus semisulcatus* de Haan. *Biol. Bull.*, 183: 394 - 400.

* Shaikhmahmud, F. S. and Tembe, V. B., 1958. Study of prawns. The reproductive organs of *Parapenaeopsis stylifera* (M. Edw). *J. Univ. Bombay*, 23: 99 - 110.

* Shaikhmahmud, F. S., 1961. A brief account of the changes in the developing ovary of (Penaeid prawns) *Parapenaeopsis stylifera* (M. Edw.) in relation to maturation and spawning cycle. *J. Univ. Bombay.*, 29(3 - 5): 62 - 77.

Shylaja, K., 1989. Tolerance limits of salinity, temperature, oxygen and pH by the juveniles of prawn *Penaeus indicus* H. Milne Edwards. M. Sc. Dissertation (Mariculture), Cochin Univ. Science and Technology.

Solis, N. B., 1988. Biology and ecology. *In:* Biology and culture of *Penaeus monodon*. SEAFDEC Aquaculture Dept., pp. 1 - 36.

* Souty, C. and Picaud, J. L., 1981. Vitellogenin synthesis in the fat body of marine crustacean Isopoda, *Odotea balthica basteri*, during vitellogenesis. *Reprod. Nutr. Dev.*, 21: 95 - 101.

Spaziani, E., 1988. Serum high density lipoprotein in the crab, *Cancer antennarius* Stimpson: II Annual Cycles. *J. Exp. Zool.*, 246: 315 - 318.

Staples, D. J., 1980. Ecology of juvenile and adolescent banana prawns, *Penaeus merguiensis* in a mangrove estuary and adjacent offshore waters of the Gulf of Carpentaria . II. Emigration, population structure and growth of juveniles. *Aust. J. Mar. Freshwater Res.*, 31: 653 - 665.

Staples, D. J., Vance, D. J. and Heales, D. S., 1984. Growth and productivity of juvenile banana prawn *Penaeus merguiensis* in natural and laborotaty systems. *In*: Proceedings of the first International Conference on the culture of Penaeid shrimps/ Prawns. (ed. Taki, Y., Primavera, J. H. and Lloberra, J. A.). SEAFDEC, Iloilo, pp. 167.

Staples, D. J. and Vance, D. J., 1987. Comparative recruitment of the banana prawn, *Penaeus merguiensis*, in five estuariesw of the south-eastern Gulf of Carpentaria, Australia. *Aust. J. Freshwater Res.*, 38: 29 - 45.

Staples, D. J. and Heales, D. S., 1991. Temperature and salinity optima for growth and survival of juvenile banana prawns *Penaeus merguiensis*. *J. Exp. Mar. Biol. Ecol.*, 154: 251 - 274.

Subrahmanyam, C. B., 1963. A note on the annual reproductive cycle of the prawn, *Penaeus indicus* (M. Edw.) of Madras coast. *Curr. Sci.*, 32(4): 165 - 166.

Subrahmanyam, C. B., 1965. On the reproductive cycle of *Penaeus indicus* (M Edwards). *J. Mar. Biol. Assoc. India*, 7: 284 - 290.

Subramoniam, T., 1999. Endocrine regulation of egg production in economically important crustaceans. *Curr. Sci.*, 76(3): 350 - 360.

Suzuki, S., 1987. Vitellins and vitellogenins of the terrestrial isopod, *Armadillidium vulgare*. *Biol. Bull.*, 173: 345 - 354.

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

Talbot, P., 1981. The ovary of the lobster, *Homarus americanus*. I. Architecture of the mature ovary. *J. Ultra. Res.*, 76: 235 - 248.

Tan, J. D., Pudadera, R. A., De Jesus, E. G. and Taki, Y., 1985. Characterization of ovarian maturation stages in wild unablated *Penaeus monodon*. *In*: Proceedings of the first International Conference on the culture of Penaeid shrimps/ Prawns. (ed. Taki, Y., Primavera, J. H. and Lloberra, J. A.). SEAFDEC, Iloilo, pp. 179.

Tan-Fermin, J. and Pudadera, R. A., 1989. Ovarian stages of the wild giant tiger prawn, *Penaeus monodon* Fabricius. *Aquaculture*, 77: 229 –242.

Tan-Fermin, J. D., 1991. Effects of unilateral eyestalk ablation on ovarian histlolgy and oocyte size frequency of wild and pond reared *Penaeus monodon* (Fabricius) broodstock. *Aquaculture*, 93: 77 - 86.

Tata, J. R., 1978. Induction and regualtion of vitellogenin synthesis by estrogen. *In*: Biochemical action of hormones, Acad. Press. New York, 5: 397 - 431.

Tata, J. R. and Smith, D. F., 1979. Vitellogenesis: A versatile model for hormonal regulation of gene expression. *Rec. Prog. Hor. Res.*, 35: 47 - 95.

Teshima, S. and Kanazawa, A., 1971. Biosynthesis of sterols in the lobster, *Panulirus japonica*, the prawn, *Penaeus japonicus*, and the crab, *Portunus tritubercalatus*. *Comp. Biochem. Physiol.*, 38B: 597 - 602.

Thongrod, S. and Boonyaratpalin, M., 1998. Cholesterol and lecithin requirement of juvenile banana shrimp, *Penaeus merguiensis*. *Aquaculture*, 161(1 - 4): 313 - 319.

Tinsley, D., 1985. A comparison of plasma levels of phosphoprotein, total protein and total calcium as indirect indices of exogenous vitellogenesis in the crucian carp, *Carassius carassius* (L.). *Comp. Biochem. Physiol.*, 80B(4): 913 - 916.

Tom, M., Goren, M. and Ovadia, M., 1987. Localization of the vitellin and its possible precursors in various organs of *Parapenaeus longirostris* (Crustacea, Decopoda, Penaeidae). *Int. J. Invert. Reprod. Dev.*,12: 1 - 12.

Tom, M., Fingerman, M., Hayes, T. K., Johnson, V., Kerne, B. and Lubzens, E., 1992 . A comparative study of the ovarian protein from two penaeid shrimps, *Penaeus semisulcatus* De Haan and *P. vannamei* (Boone). *Comp. Biochem. Physiol.*, 102B: 483 - 490.

Trujillo, L.R and Primavera , J. H., 1986. Induced maturation in ablated *Penaeus notialis* and *Penaeus schmitti*. *In:* Proceedings of the First Asian Fisheries Forum (ed. Maclean, J. L.; Dizon, L. B.; Hosillos, L. V.). Manila, Philippines, pp. 697-700

Tsukimura, B. and Kamemoto, 1991. In vitro stimulation of oocyte by presumptive mandibular organ secretions in the shrimp, *Penaeus vannamei*. *Aquaculture*, 92: 59 - 66.

Tsuzuki, M. Y. and Cavalli, R. O., 2000. The effects of temperature, age and acclimation to salinity on the survival of *Farfantepenaeus paulensis* postlarvae. *J. World Aquacul. Soc.*, 31(3): 459 - 468.

Tuma, D. J., 1967. A description of the development of primary and secondary characters in the banana prawn, *Penaeus merguiensis* de Man (Crustacea: Decapoda: Penaeinae). *Aust. J. Mar Freshwat. Res.*, 18: 73 - 88.

Vaca, A. A. and Alfaro, J., 2000. Ovarian maturation and spawning in the white shrimp, *Penaeus vannamei* by serotonin injection. *Aquaculture*, 182: 373 - 385.

Van-Herp, F., 1988. Inhibiting and stimulating neuropeptides controlling reproduction in Crustacea. *Invert. Reprod. Dev.*, 22: 21 - 30.

Van-Herp, F. and Soyez, D., 1997. Reproductive biology in invertebrates: Arthropoda-Crustacea. *In:* Progress in Reproductive Endocrinology, Part A, (ed. Adams T. S.). Oxford and IBH, New Delhi, pp. 247 - 275.

Varadarajan, S. and Subramoniam, T., 1980. Biochemical changes during vitellogenesis in a hermit crab *Clibanarius clibanarius*. *In*: Progress in Invertebrate Reproduction and Aquaculture, Proceedings of the First All India Symposium on Invertebrate Reproduction (ed. Subramoniam, T. and Varadarajan, S.). Indian Society of Invertebrate Reproduction, Madras, pp. 7 - 14.

Varghese, B., 1999. Effect of salinity on survival, growth and biochemical components of *Penaeus monodon* Fabricius postlarvae. M. Sc. Dissertation, CIFE, Mumbai.

Vasudevappa, C., 1992. Growth and reproduction of the penaeid prawn *Metapenaeus dobsoni* (Miers) in brackishwater environment. Ph. D. Thesis, Cochin University of Science and Technology

Vazquez-Boucard, C., Ceccaldi, H. J., Benyamin, Y. and Roustan, C., 1986. Identification, purification et characterization de la lipovitelline chez un Crustace Decapode Natantia *Penaeus japonicus* Bate. *J. Exp. Mar. Biol. Ecol.*, 97: 37 - 50.

Venkataramaiah, A., Lakshmi, G. J. and Gunter, G., 1972. The effects of salinity and feeding levels on the growth rates and food conversion efficiency of the shrimp *Penaeus aztecus*. In Proc. Third Workshop, World Maricul. Soc., pp.267 - 283.

Vijayan, K. K. and Diwan, A. D., 1995. Influence of temperature, salinity, pH and light on moulting and growth in the Indian white prawn *Penaeus indicus* under laboratory conditions. *Asian Fisheries Sci.*, 8(1): 63 - 72.

Vinod, K., Bhat, U. G. and Neelakantan, B., 1996. Effect of salinity and food intake, growth and conversion efficiencies of juvenile *Penaeus merguiensis (de Man). Environ. Ecol.*, 14(1): 71 - 75.

Wallace, R. A., Walker, S L. and Hausehka, P. V., 1967. Crustacean lipovitellin: isolation and characterisation of the major high density lipoprotein from the eggs of decapods. *Biochem.*, 6: 1582 - 1590.

Wallace, R. A. and Bergink, W. E., 1974. Amphibian vitellogenin: Properties, hormonal regulation of hepatic synthesis and ovarian uptake and conversion to yolk proteins. *Am. Zool.*, 14: 1159 - 1175.

Wallace, R. A., 1978. Oocyte growth in non-mammalian vertebrates. *In:* The Vertebrate Ovary (ed. Jones, R. E.). Plenum, New York, pp. 469 - 502.

Wallace, R. A., 1985. Vitellogenesis and oocyte growth in non-mammalian vertebrates. *In:* Developmental biology: A comprehensive synthesis, (ed. Browder, L. W.). Plenum Press, New York, pp. 127 - 177.

Wear, R, G. and Santiago, A., Jr., 1976. Induction of maturity and spawning in *Penaeus monodon* Fabricius by unilateral eyestalk ablation (Decapoda: Natantia). *Crustaceana*, 31: 218 - 220.

Wilder, M. N. and Aida, K. ,1995. Crustacean ecdysteroids and juvenoids: chemistry and physiological roles in two species of prawn, *Macrobrachium rosenbergii* and *Penaeus japonicus*. *Israeli J. Aquacul., Bamidgeh*, 47: 129 - 136.

Wilder, M. N., Okada, S., Fusetani, N. and Aida, K., 1995. Haemolymph profile of juvenoid substance in the giant freshwater prawn *Macrobrachium rosenbergii* in relation to reproduction and moulting. *Fish. Sci.*, 61 (1): 115 - 120.

Wolin, E. M., Laufer, H. and Albertini, D. F., 1973. Uptake of the yolk protein, lipovitellin, by developing crustacean oocytes. *Dev. Biol.*, 35: 160 - 170.

Wyban, J. A. and Sweeney, J. H., 1991. Intensive shrimp production technology. *In:* The Oceanic Institute Shrimp Manual, The Oceanic Institute, Honolulu, pp. 1 -11.

Yano, I., 1985. Induced ovarian maturation and spawning in greasyback shrimp, *Metapenaeus ensis*, by progesterone. *Aquaculture*, 47: 223 - 229.

Yano, I., 1987. Effect of 17 alpha-hydroxyprogesterone on vitellogenin secretion in kuruma prawn, *Penaeus japonicus*. *Aquaculture*, 61(1): 49 - 57

Yano, I. and Chinzei, Y., 1987. Ovary is the site of vitellogenin synthesis in kuruma prawn, *Penaeus japonicus*. *Comp. Biochem. Physiol.*, 86B (2): 213 - 218

Yano, I., 1988. Oocyte development in the kuruma prawn *Penaeus japonicus* . *Mar. Biol.*,99 (4): 547 - 553

Yano, I., Tsukimura, B., Sweeney, J. N. and Wyban, J. A., 1988. Induced ovarian maturation of *Penaeus vannamei* by implantation of lobster ganglion. *J. World Aquacult. Soc.*, 19 (4): 204 - 209

Yano, I., 1992. Effect of thoracic ganglion on vitellogenin secretion in kuruma prawn, *Penaeus japonicus*. *Bull. Natl. Res. Inst. Aquacult.*, 21: 9 - 14

Yano, I. and Wyban, J. A., 1992. Induced ovarian maturation of *Penaeus vannamei* by injection of lobster brain extract. *Bull. Natl. Res. Inst. Aquacult.*, 21: 1-7

Yano, I., 1993. Ultraintensive culture and maturation in captivity of penaeid shrimp *In:* CRC Handbook of Mariculture Vol. 1: Crustacean Aquaculture (ed. McVey, J. P.), CRC Press, Boca Raton, FL, USA, pp. 289-313

Yap, W. G., 1999. Shrimp culture: a global overview. SEAFDEC Asian Aquaculture, 21 (4): 18 - 37.

Young, N. J., Quinlan, P. T. and Goad, L. J., 1992. Progesterone metaobolism in vitro in the decapod crustacean, *Penaeus monodon*. *Gen. Comp. Endocrinol.*, 87(1-3): 300 - 311.

Zein- Eldrin, Z. P., 1963. Effect of salinity on growth of postlarval penaeid shrimp. *Biol. Bull.*, 125(2): 188 - 196.

Zein - Eldin, Z. P. and Griffith, G. W., 1968. An appraisal of the effects of salinity and temperature on growth and survival of postlarval penaeids. FAO Fisheries Report, 57: 1015 - 1026.

* Not referred in original