BIOCHEMICAL CHANGES DURING MATURATION IN THE MARINE SHRIMP *Parapenaeopsis stylifera* (H. MILNE EDWARDS)

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JULY 1998

POST GRADUATE PROGRAMME IN MARICULTURE
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
COCHIN - 682 014

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DISSERTATION SUBMITTED BY

KRISHNA SUDHA T.

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Oh dear ! Can't Believe

You are no more with me

You passed away silently

With no words to console

Don't know what you suffered

Nobody told the truth

Somebody took her Away

But won't believe the truth !!

Yet Feel Your Blessings Mom

And still refuging under your wings

DEDICATED TO

MY BELOVED MOTHER

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CERTIFICATE

Certified that the dissertation entitled "BIOCHEMICAL CHANGES DURING MATURATION IN THE MARINE SHRIMP *Parapenaeopsis stylifera* (H. MILNE EDWARDS)" is a bonafide record of the work done by Miss KRISHNA SUDHA. T. under our guidance at the Central Marine Fisheries Research Institute during the tenure of her M.F.Sc. (Mariculture) programme of 1995-1998 and that it has not previously formed the basis for the award of any other degree, diploma or other similar titles for any other publication.

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DECLARATION

I hereby declare that this thesis entitled "BIOCHEMICAL CHANGES DURING MATURATION IN THE MARINE SHRIMP *Parapenaeopsis stylifera* (H. MILNE EDWARDS)", is based on my own research and has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles of recognition.

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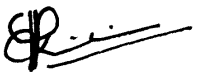
July, 1998.



(KRISHNA SUDHA. T)

सारांश

समुद्री झींग पी.स्टाइलिफेरा की परिपक्वता की विभिन्न अवस्थाओं में प्रोटीन, कार्बोहाइड्रेट, लिजिड और लवणता की सांद्रता में होने वाले परिवर्तन का अध्ययन किया गया । इसमें रूधिरलसीका (हीमोलिम्फ) और पेशी, यकृतन्याश्य (हेपाटोपान्क्रियास) और अंडाशध के विभिन्न उतकों का अध्ययन भी शामिल है । रूधिरलसीका के प्रोटीन के अंश में पहली अवस्था से वौधी अवस्था तक क्रमिक वृद्धि देखी गयी और चौधी अवस्था में अधिकतम मापन ।05.29 मि ग्र/मि लि आकलित किया गया । अंडाशय के प्रोटीन का स्तर भी पहली अवस्था से चौथी अवस्था तक क्रमिक रूप से बढ गया और चौधी अवस्था में यह 42.59 मि ग्रा/।00 मि ग्र आकलित किया गया । पेशी के प्रोटीन का स्तर आंघत ही था । यकृतगन्याशय के प्रोटीन का अंश सामान्य रूप से कम देखा गया (9.।8 मि ग्रा/।00 मि ग्रा से ।3.76 मि ग्रा/।00 मि ग्रा) ।

रूधिरलसीका के कार्बोहाइड्रेट के कार्बोहाइड्रेट का स्तर चौथी अवस्था में उच्चतम (2.20 मि ग्रा/ मि लि) और पहली अवस्था में निम्नतम (0.49 मि ग्रा/मि लि) था । अंडाशय के कार्बोहाइड्रेट की मात्रा में चौथी अवस्था तक क्रमिक वृद्धि दिखाई पडी और इस अवस्था में अधिकतम स्तर (3.0। मि ग्रा/।00 मि ग्रा) दिखाई पडा । पेशी में कार्बोहाइड्रेट के स्तर में उतार-पढाव देख गया । यकृतगन्याशय में अधिकतम कार्बोहाइड्रेट दिखाया पडा जो तीसरी अवस्था में चरम सीमा पर था और घट गया ।

चौथी अवस्था में रूधिरलसीका में लिजिड का स्तर उच्चतम (23.78 मि ग्रा/मि लि) और पहली अवस्था में निम्नतम देखी गई 8.2। मि ग्रा/मि लि) अंडाशय के लिजिड स्तर में पहली से चौथी अवस्था तक अधिक वृद्धि (।3.।0 मि ग्रा/।00 मि ग्रा से 32.23 मि ग्रा/।00 मि ग्रा तक) और पाँचवीं अवस्था में घटती (।3.।0 मि ग्रा/।00 मि ग्रा) देखी गई । पेशी के उतकों में लिजिड बहुत कम मात्रा में देखे गए । तीसरी अवस्था में यकृतागन्याशय में लिजिड की मात्रा अधिकतम (59.37 मि ग्रा/।00 मि ग्रा) थी । चौथी और पाँचवीं अवस्थाओं में इसमें क्रमिक ह्रास दिखाया पडा ।

अंडाशय की नमी की मात्रा में उल्लेखनीय उतार-चढाव देख गया । पेशी में लवणता का अंश परिपक्वता की विभिन्न अवस्थाओं में 74.56/ से 78.62/ की बीच था । यकृतगन्याशय में नमी की मात्रा तीसरी अवस्था में बहुत कम (52.06/ रही । तीसरी से चौथी अवस्था तक आते - आते इसमें बढोतरी की प्रवणता देखी गई ।

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***

PREFACE

Crustaceans occupy an important position among the fishery resources all over the world. A survey of the literature on the biochemistry of crustaceans revealed that significant inter-specific variations exist in the biochemical composition of tissues. Ovarian maturation places enormous demands on the energy reserves of females. A gametogenic process in the female reproductive cycle of crustaceans includes synthesis of nutritive yolk in the ooplasm to meet the basic requirements of embryonic development independent of the maternal organism. During the process, considerable mobilisation takes place in the major organic reserves of the animals.

The hepatopancreas or the midgut gland has been identified as the primary organ responsible for the storage of organic reserves in crustaceans, whereas haemolymph plays the role of transporting these metabolites to different tissues. The glycogen and lipid are the major energy reserves affected at the time of reproduction in crustaceans. However, information on quantitative and qualitative changes in these organic reserves is scanty, especially with reference to reproduction.

Studies on biochemical changes in reproductive cycle in invertebrates was initiated in 1974. Among crustaceans, most of these studies are centred on the changes in the metabolites like protein, lipid and carbohydrates in relation to different stages

of maturity in pleocyematans like Portunus pelagicus, Charybdis variegata, Paratelphusa hydrodromous, Uca annulipes, P.pelagicus, Barytelphusa cunicularis, Orconectes nais, Menippe rumphii and Clibanarius clibanarius. Similar studies on dendobranchiate crustaceans are few, although the annual reproductive cycle of penaeid shrimps has been well documented. The fluctuations in the biochemical constituents such as water, protein, lipid and carbohydrates in gonad, muscle, hepatopancreas in relation to different reproductive phase has been investigated in penaeid shrimp, Metapenaeus affinis. Similar studies have been carried out on open thelycum penaeid such as P.vannamei, P.stylirostris and P.setiferus. The levels of protein, glycogen and fat in the hepatopancreas and ovary were studied in relation to maturation in the marine shrimp, Parapenaeopsis hardwickii, P.indicus and Metapenaeus dobsoni.

Proteins provide the basic structural material for tissue build up. Studies on haemolymph protein content in relation to ovarian maturation in crustaceans are limited. Vitellogenin or the plasma precursor of yolk protein has been studied in crabs and crayfish. The appearance of vitellogenin in the blood sera has been studied in P.japonicus. Lipovitellin which is the principal fraction of the crustacean vitellus has been investigated in relation to maturation of the ovary in a number of crustaceans. The accumulation and cyclic variation in protein content of the gonad, hepatopancreas and muscle has been studied in M.affinis, P.hardwickii and in P.setiferus.

Informations on the fluctuations in free sugar (glucose, galactose and sucrose) in the hepatopancreas of the crab, P.hydrodromous in relation to ovarian cycle is available. In P.hardwickii studies have been made on the proportionality between haemolymph glucose level and ovary growth. The variations in the glucidic metabolism during the reproductive cycle has been studied in P.notalis.

The chief storage site of lipid in crustaceans is found to be the hepatopancreas and the quantitative and qualitative composition of lipid detected at any given time in this organ has been the result of absorptive, synthetic, secretory and catabolic processes. Due to the enormous quantity of lipid involved in crustacean vitellogenesis, several workers have investigated the changes in the fat content of the gonad and hepatopancreas. Relatively few studies have been carried out on haemolymph lipids in crustaceans. The metabolism of lipids in relation to reproduction has been studied in the freshwater crab, P.hydrodromous. Literature on the maturational changes in the ovarian lipid spectrum of the pink shrimp P.duorarum and metabolic profile of dietary lipids in relation to ovarian maturation in penaeid shrimps has been well documented. There are also studies on the variation in lipid composition during ovarian maturation and in tissue lipid composition during vitellogenesis in P.indicus.

Crustaceans exhibit a general pattern in the fluctuation of metabolic components, but significant variations are known to

occur at intrageneric and intraspecific levels. Therefore, a separate study on each species is essential for understanding the precise body metabolism in conjunction with the biosynthesis of yolk in the ovary.

In the export trade of India, shrimps play a dominant role. The penaeid shrimp, Parapenaeopsis stylifera contributes significantly to capture fisheries in India. It is most abundant from Veraval to Trivandrum coast, moderate in the northern and southernmost regions on the west coast and sparse along east coast. P.stylifera is stenohaline and spends its complete life cycle in the sea unlike most of the penaeid shrimps. All stages including juveniles are seen in inshore areas. It grows upto 90-100 mm in first year. Size at first maturity is 65 mm in males and 70 mm in females. It spawns round the year, peak being December-May in Kerala.

In the present study on female P.stylifera, the quantitative variations in protein, carbohydrates, lipid, and moisture in the various tissues like ovary, hepatopancreas, muscle and haemolymph has been studied during different stages of maturity. The results of the investigation will help us to gain an understanding of the potential store house of various metabolites and to trace the pathways though which they are mobilized for biological needs of the shrimp.

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INTRODUCTION

Gonadal growth in invertebrates in the pre-spawning period is an elaborate process, involving active mobilisation and synthesis of organic substances. Development of Gonad represents a remarkable synthesis of organic material (Giese et al, 1958), gonad being the locus of intensive biochemical synthesis at the time of formation of gametes. Reproduction in shrimp like in other crustaceans is a dynamic process mediated by several endogenous and exogenous factors which influence the development and formation of gametes, their maturation and subsequent release. Among the endogenous factors, mobilisation of major nutrients and other biochemical constituents for the gamete formation and maturation are important (Giese et al 1958). Scientific investigations on the biology of shrimp dates back to the middle of 14th century. Since then, a wealth of information has been generated about commercially important species whose synopsis has been published in the Proceedings of World Scientific Conference on the "Biology & Culture of shrimps and prawns" held at Mexico in 1967 (Mistakidis, Ed. 1968, 1969, 1970). Subsequent to this, several other workers also have added to our knowledge on the notable contributions being those by Jones (1961), Rajalakshmi (1961), Subramanyan (1965, 1973), Ramakrishnan (1979); George et al (1967), Rao (1968); Pillai & Nair, (1973), Kuttyamma (1973); Kurup & Rao (1974, 1989); George & Rao, (1974), Thomas (1974), Muthu and Laxminarayana (1977),

Ramamurthy *et al* (1978), Kagwade (1980), Suseelan & Kathirvel, (1982a), Kagwade (1980), Achuthankutty & Nair (1983), Laxminarayana & C.S. Sasidharan (1985), Sukumaran *et al*, (1987) Lalitha Devi (1987) & Laxminarayana (1987).

Changes in biochemical constituents are pronounced in invertebrates which are cyclic in reproduction, since a great amount of energy must be chanelled to the gonads during reproduction. This is reflected in deposition or depletion of nutrients with the advent or departure of the reproductive period (Cambert & Dahnel, 1974). The predominant organic reserves of many crustaceans are glycogen and lipids (O'connor & Gilbert, 1968) which are accumulated by midgut gland and ovaries during gonadal development. It has been shown that organic material may be transferred from midgut gland to the gonad as animal matures. Particularly glycogen may serve as reserve food material to be utilized for the formation of gonadal elements

Although extensive work is available on the changes in the various body components of crustaceans in relation to moulting cycle, studies on biochemical changes occurring during maturation of gonads are rather scanty (Sastry, 1983). Some of the earlier noteworthy contributions in the field were by George & Patel (1956) on fresh water decapodes, Barnes *et al* (1963) on *Balanus balanus*, Dean & Vernberg (1965) on *Callinectus sapidus*, Heath & Barnes (1970) on *Carcinus maenas*, Pillay & Nair (1972) on *Balanus amphitrite* & Diwan and Nagabushanam (1974) on

Barytelphusa cunicularis, Varadarajan and Subramaniam (1982) on Clibanarius clibanarius. The contributions of Yano & Chinzei (1987) on Penaeus japonicus and reviews by Giese & Pierse (1974) & Adiyodi & Subramoniam (1983) covered this important aspect of biochemical changes related to reproduction.

Variations in concentration of major organic reserves of yolk, like protein, lipid and carbohydrates in relation to different stages of maturity in various groups of crustaceans have been fairly well studied (Barnes et al; 1963; Pillai & Subramaniam, 1982). Hepatopancreas or midgut gland has been identified as the major organic reserve organ in crustaceans (Chang & O'Connor, 1983). Adiyodi & Subramoniam (1983) in their review on crustacean reproduction stressed the importance of hepatopancreas as a storage depot and haemolymph as a transport media of nutrients during maturation of ovary specially during vitellogenesis. Yano (1988) investigated appearance of vitellogenin in the blood sera of mature female P.japonicus. Sunilkumar (1989) studied changes in haemolymph proteins in relation to maturation in P.indicus. Biochemical variations in the haemolymph different maturity stages has been studied by Laxmilatha (1991) in P.indicus and Vasudevappa in Metapenaeus dobsoni (1992) and Joshi (1991) in M.idella.

Uptake of hepatopancreatic lipid during maturation is described in the crab, P.hydrodromus (Anilkumar, 1980) & C.clibanarus (Varadarajan & Subramoniam, 1982). Similar findings

have been reported by Kulkarni and Nagabhushanam (1979) in P.hardwickii & Sunilkumar (1989) in P.indicus.Contradicting the above reports, Galois(1984) reported opposite trend in P.indicus. Some reports are available indicating the mobilization of muscle lipid during maturation in the crab C.longitarsus (Ajmalkhan & Natarajan, 1980). Inverse relationship between water content and gonad development have been reported in M.affinis and Portunus pelagicus (Pillay & Nair, 1973) and in P.indicus (Sunilkumar, 1979). Variations in lipid profile of ovary and hepatopancreas during maturation in P.japonicus has been delineated by Teshima et al (1989). Biochemical changes in Indian species have been reported by Kulkarni & Nagabhushanam (1979) in P.hardwickii, Achuthankutty & Parulekkar (1984) in M.affinis; M.dobsoni, P.merquiensis and P.stylifera and Mohammed and Diwan (1992) in P.indicus.

Different immunoelectrophoretic forms of yolk proteins marking different stages of maturity cycle in stomatopods by Marzari, R. et al, (1993), changes in concentration of female specific high density lipoproteins in hepatopancreas, ovaries and different stages of blue crab Callinectes sapidus by Lee et al, (1991) are some of recent studies in this field.

Review of literature reveals that among crustaceans, studies on biochemical changes related to gonad growth have concentrated mostly on brachyurans. Crustaceans specially of the genes Parapenaeopsis has been given very little attention. Hence

in the present study, it was felt necessary to study biochemical changes in haemolymph, muscle, hepatopancreas and ovary during various maturity stages of female, P.stylifera which is an important shrimp in capture fisheries. Quantitative variations of protein, lipid, carbohydrate and moisture content in various tissues during five maturity stages are investigated.

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FIG. 1. *Parapenaeopsis stylifera*


20 21 22 23 24 25 26 27 28 29 30 31

MATERIALS AND METHODS

The marine shrimp *Parapenaeopsis stylifera* H.Milne Edwards was selected for the present study. Live females of *P.stylifera* were collected by trawlnet operations off Cochin. The live females were brought to the laboratory and kept in FRP tanks containing seawater (32-33 ppt) with continuous aeration. The females were then segregated to the five maturity stages based on the size, shape, colour of the ovary and microscopic details of ova as presented below:

Stage I - Immature: Ovary thin, translucent and strandlike, not visible through the exoskeleton; anterior and middle lobes confined to the posterior half of cephalothorax, eggs spherical with conspicuous necleus and clear cytoplasm.

Stage II - Early maturing: Ovary moderately enlarged. Light yellowish green in colour; anterior and middle lobes faintly visible through exoskeleton, anterior lobe extending up to base of the rostrum, lobules of middle lobe partly covering posterior region of hepatopancreas, abdominal lobe not clearly visible through exoskeleton; eggs oval or spherical, opaque, cytoplasm with sparsely distributed yolk granules and vacuolated.

Stage III - Late maturing: Ovary fairly large. Light green in colour and clearly visible through exoskeleton throughout its length; anterior lobe extending beyond base of rostrum, lobules of middle lobe further enlarged and covering the whole dorsal

side of hepatopancreas, diamond shaped enlargement of posterior lobe clearly visible through exoskeleton, eggs oval or spherical, opaque cytoplasm embedded with yolk all over.

Stage IV - Mature or ripe: Ovary fully enlarged dark green in colour and clearly visible through exoskeleton, anterior lobe and middle lobe occupying the entire cephalothoracic region, diamond-shaped enlargement of posterior lobe in first abdominal segment clearly noticeable, egg fully embedded with yolk.

Stage V - Spent/spent recovering: Ovary loose and flaccid, translucent, anterior lobe slightly retracted, middle and posterior lobe not visible through exoskeleton; most of the ova small and spherical with clear cytoplasm and nucleus, residual eggs in early and late maturity stages seen at various stages of reabsorption.

Collection of haemolymph and sampling of tissue

Live shrimps were collected and blotted dry using a filter paper. Haemolymph samples from individual female shrimps were drawn by direct cardiac puncture using hypodemic syringe fitted with a No.22 needle. The glass syringe and needle used for haemolymph collection were rinsed in an anti-coagulant (10% trisodium citrate) prior to each collection. The collected haemolymph samples were stored in sterilized glass vials at $-20^{o}C$ until analysis. After the extraction of the haemolymph, the females were immediately dissected and the ovary, hepatopancreas and muscle tissues were quickly excised out. Tissues were dried

to a constant weight at 60°C and then mascerated using a mortar and pestle. They were then stored in a dessicator with silica gel until analysis. Replicates were carried out for each estimation.

BIOCHEMICAL ANALYSIS

1. Estimation of moisture content

The moisture content of ovary, hepatopancreas and muscle was determined by keeping pre-weighed wet samples at 60°C in a hot air oven till constant weights were obtained. Percentage moisture in the samples were calculated as follows:

$$\text{Percent moisture} = \frac{\text{Difference in wet weight - dry weight of samples}}{\text{wet weight of tissue}} \times 100$$

Standard methods were followed for all biochemical estimations with necessary modifications. Standard graphs were plotted with the concentration of each biochemical parameter in different dilutions of the working standard solution, in the X-axis, the optical density in the Y-axis. Concentration of different parameters in the samples was compared and calculated from the graph also by the following formula.

$$\text{Amount of biochemical constituent present in the sample} = \frac{\text{OD of sample}}{\text{OD of the std.}} \times \frac{\text{concentration of the Std}}{\text{weight of the sample}}$$

Total optical density or the colour developed for total proteins, total free amino acids, glucose, total lipids and

cholesterol was measured using a spectrophotometer. Samples were taken in quartz cuvettes. Only analar grade, extrapure chemicals were used for all estimations.

2. Estimation of total protein

Total proteins was estimated by the method of Lowry et al (1951) using bovine, serum albumin (Sigma) as standard. Pre-weighed fresh tissue or a known aliquot of haemolymph was taken for estimation. Results were calculated as percent wet weight of the tissue based on standard graph of transmittance at 540 nm.

3. Estimation of total carbohydrates

Total carbohydrates were estimated using the phenol sulpuric acid method of Dubois et al (1956). The tissue samples were deproteinized using 80% ethanol. To a known aliquot of the supernatent 0.5 ml of phenol reagent was added. Then 2.5 ml of concentrate H_2SO_4 was added directly against the liquid surface to obtain good mixing. The solution after thorough shaking was allowed to stand at $30^{o}C$ in a waterbath for 20 minutes. The optical density of the coloured solution was read at 490 nm in a UV-VIS Spectrophotometer along with D-glucose standard and reagent blanks.

4. Estimation of total lipids

Total lipids were estimated by Sulphophosphovanillin method of Barnes and Blackstock, (1978). 2g vanillin in 1 litre of Orthophosphoric is kept in dark bottle at room temperature. Standard Cholesterol is prepared by taking 80 mg of cholesterol

in 100 ml 2:1 chloroform Methanol (2:1 V/V) mixture which is equivalent to 100 mg of total lipid in 100 ml 2:1 V/V Chloroform Methanol mixture. 50-500 ug/0.5 ml is taken as working standard. 0.5 ml of Chlorofarm Methanol is treated as blank.

(1) To 100 μl of haemolymph 1 ml of Chloroform Methanol mixture was added and left overnight in a refrigerator.

(2) 0.5 ml of this lipid extract was taken in clean, dry glass tubes and dried in vacuom over silica gel in dessicator.

(3) To the dried sample, 0.5 ml of concentrated H_2SO_4 was added and shaken well.

(4) Tube was plugged with non-absorbant cotton and heated at 100^{o}C in a boiling water bath for 10 mts and cooled under running water.

(5) 0.1 ml of the acid digested is transferred to a clean, dry, glass tube and 2.5 ml of sulphophosphovanillin reagent was added and mixed in a cyclomixer.

(6) After 30 minutes, O.D. of pink red colour developed is measured at 520 nm against blank.

Statistical Analysis of data:

The mean and standard deviations of the data were determined and values were plotted on graphs to obtain the trend of metabolites during different maturity stages of shrimps. Analysis of variance (ANOVA) was performed to test the significance between treatments i.e the effect of different maturity stages on various biochemical parameters (Snedecor and Cochran, 1968).

***

RESULTS

The data on estimated protein, carbohydrates, lipid and moisture content in the muscle, haemolymph, hepatopancreas and ovary at different stages of maturity are presented in Table 1 to 4 and are represented graphically in Figs. 2 to 16.

1. Proteins:-

The variations in the concentration of total proteins in the haemolymph, ovary, muscle and hepatopancreas in different maturity stages are given in Table 1 and are depicted graphically in figures 2 to 5.

The protein content in the haemolymph increased gradually from stage 1 to stage IV, where the maximum value of 105.29 mg/ml was observed. In Stage V, there was a sharp fall in the haemolymph protein content to 40.46 mg/ml (Fig.2). The levels of protein in the ovary gradually increased from stage I to IV and the maximum value of 42.59 mg/100 mg was observed in stage IV. Spent ovaries displayed a sudden decrease in protein content (24.28mg/100 mg) as shown in Fig.3. Muscle protein content was uniformly high in all maturity stages and the values showed an erratic behaviour without a definite pattern (Fig.4). The protein content in hepatopancreas was found to be generally poor (9.18 mg/100mg - 13.76 mg/100mg). Maximum protein content was observed in stage I and V and minimum value of 9.18 mg/100 mg was obtained in stage III (Fig.5).

TABLE 1 - VARIATIONS IN CONCENTRATION OF TOTAL PROTEIN DURING DIFFERENT MATURITY STAGES OF P.STYLIFERA

Tissue	Maturity stages				
	I	II	III	IV	V
Haemolymph (mg/ml)	32.72 ±1.019	50.49 ±1.289	74.68 ±1.053	105.29 ±4.539	40.46 ±1.537
Ovary (mg/100 mgdw)	18.34 ±1.441	25.60 ±1.315	34.94 ±2.141	42.59 ±1.582	24.28 ±1.517
Muscle (mg/100 mgdw)	53.54 ±0.579	53.10 ±1.149	57.83 ±2.335	51.14 ±1.565	52.80 ±1.047
Hepato-pancrease (mg/100 mgdw)	13.76 ±0.737	11.49 ±0.632	9.18 ±0.508	12.26 ±4.946	14.00 ±0.478

ANALYSIS OF VARIANCE - PROTEIN

Source	df	SS	MS	F
Tissue	3	37613.513	12537.838	3851.148**
M.stage	4	7184.942	1796.236	551.735**
Interaction	12	12261.09	1021.757	313.845
Error	80	260.449	3.256	-
Total	99	57319.994	578.990	-

** Highly significant

ANOVA showed that the difference in protein levels in haemolymph, ovary, muscle and hepatopancreas during the different maturity stages were statistically significant at 1% level.

2. Carbohydrates:-

The variations in total carbohydrates in the haemolymph, ovary, muscle and hepatopancreas during the different maturity stages are given in Table 2. In the haemolymph, the total carbohydrate content was the lowest in stage I (0.49 mg/ml). A gradual increase in the carbohydrate content was observed and the maximum value of 2.20 mg/ml was noticed in stage IV. In stage V, there was a drastic decline in the carbohydrate level (0.71 mg/ml). The carbohydrate content in the ovary showed a gradual increase from stage I to IV (2.25 mg/100 mg to 3.01 mg/100 mg) and subsequent decrease in stage V (2.12 mg/100 mg). Peak carbohydrate level of 3.01 mg/100 mg was observed in stage IV ripe ovaries. In the muscle, carbohydrate levels were generally low (1.15 mg/100 mg to 1.34 mg/100 mg) and fluctuated without a definite pattern (Fig.8). Maximum carbohydrate content was observed in the hepatopancreas. Peak levels of 4.1% was observed in stage III and thereafter there was a drastic decline in the carbohydrate content in stage IV (9.17 mg/100 mg).

ANOVA revealed that the differences in total carbohydrate content in the haemolymph, ovary, muscle and hepatopancreas were statistically significant at 1% level. The carbohydrate levels in the muscle did not show any significant variation.

Table 2. VARIATIONS IN THE CONCENTRATION OF CARBOHYDRATE DURING DIFFERENT MATURITY STAGES OF P.stylifera

Tissue	Maturity stage				
	I	II	III	IV	V
Haemolymph (mg/ml)	0.49± 0.021	0.88± 0.074	1.26± 0.033	2.20± 0.071	0.71± 0.034
Ovary (mg/100mgdw)	2.25± 0.109	2.38± 0.117	2.61± 0.048	3.01± 8.096	2.12± 0.099
Muscle (mg/100mgdw)	1.24± 0.061	1.15± 0.073	1.22± 0.056	1.31± 0.048	1.34± 0.034
Hepato-pancreas (mg/100mgdw)	2.44± 0.089	3.32± 0.068	4.19± 0.10	1.70± 0.034	2.33± 0.044

ANALYSIS OF VARIANCE - CARBOHYDRATE

Source	df	SS	MS	F
Tissue	3	54.537	18.179	2664.759**
M-Stage	4	7.293	1.823	267.265**
Interaction	12	23.155	1.930	282.843
Error	80	0.546	0.007	-
Total	99	85.530	0.864	-

** Highly significant

3. Lipids:-

The changes in lipid content in the haemolymph, ovary, muscle and hepatopancreas during the different stages of maturity are given in Table 3. High lipid levels in the haemolymph was observed in stage IV (23.78 mg/ml) while the lowest was seen in stage I (8.21 mg/ml). Haemolymph of spent female showed a sudden decline in lipid levels (Fig. 10). In the ovary, there was a drastic increase in lipid content from stage 1 to stage IV (13.10 mg/100mg to 32.23 mg/100 mg). In stage V the lipid content decreased (15.47 mg/100 mg) and was close to the level seen in stage I (13.10 mg/100 mg). Muscle tissues were found to be uniformly poor in lipids with levels ranging from 6.02 mg/100 mg to 7.85 mg/100 mg. No definite trend could be observed in the muscle lipid content in relation to maturity stages (Fig. 12). Lipid content of the hepatopancreas during the different maturity stages ranged from 14.14 mg/100 mg to 59.37 mg/100 mg to the maximum of 59.37 mg/100 mg in stage III. After this rapid increase in lipid content, there was a gradual decrease in stage IV (55.17 mg/100 mg) and in Stage V (35.78 mg/100 mg). The hepatopancreas in spent females also were remarkably rich in lipids (35.78 mg/100 mg).

ANOVA indicates that the variations in lipid levels in the haemolymph, ovary and hepatopancreas during the different maturity stages were statistically significant at 1% level. Changes in the muscle lipid content was not statistically significant.

TABLE 3. VARIATION IN THE CONCENTRATION OF LIPID DURING DIFFERENT MATURITY STAGES OF P.STYLIFERA

Tissue	Maturity stages				
	I	II	III	IV	V
Haemolymph (mg/ml)	8.21± 0.121	12.59± 2.175	17.20± 0.794	23.78± 0.808	15.75± 0.729
Ovary (mg/100mgdw)	13.10± 0.639	17.93± 0.847	25.78± 1.271	32.23± 1.343	15.47± 1.419
Muscle (mg/100mgdw)	6.02± 0.402	6.76± 0.206	7.40± 0.196	7.85± 0.129	7.07± 0.412
Hepato-pancreas (mg/100mgdw)	14.14± 1.196	28.54± 1.297	59.37± 1.002	55.17± 1.392	35.78± 1.322

ANALYSIS OF VARIANCE - LIPID

Source	df	SS	MS	F
Tissue	3	13361.746	4453.915	3418.291**
M-Stage	4	5135.241	1283.810	985.299**
Interaction	12	3862.298	321.858	247.020
Error	80	104.237	1.303	-
Total	99	22463.523	226.904	-

** Highly significant

4. Moisture:-

Table 4 depicts the variations in the moisture or water content of the ovary, muscle and hepatopancreas during different stages of maturity.

The moisture content in the ovary showed remarkable fluctuations with the maturity stages. Maximum water content was observed in stage V (82.56%) and stage 1 (76.24%). From 76.24% in stage I, the water content declined to 67.06% in stage IV. Spent ovaries were found to have the maximum water content (Fig.14). The moisture content in the muscle was found to vary from 74.56% to 78.62% in the different maturity stages without showing a definite pattern. In comparison to the other tissues, hepato-pancreas was observed to have the least moisture content (Table 4). In stage 1 the moisture content was 69.88% and thereafter the values showed a declining trend and the lowest value of 52.60% was recorded in stage III. From stage III to stage V, the moisture content values showed a rising trend (Fig. 16).

ANOVA showed that the percentage variations of moisture in ovary and hepatopancreas during different maturity stages were statistically significant. However, the changes in the muscle water content was not significant.

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Table 4. VARIATIONS IN THE LEVEL OF MOISTURE DURING DIFFERENT MATURITY STAGES OF P.STYLIFERA

Tissue	Maturity stages				
	I	II	III	IV	V
Ovary (%)	76.24± 1.022	71.92± 0.831	70.06± 0.845	67.06± 0.985	82.56± 0.589
Muscle (%)	77.60± 0.558	78.62± 1.156	76.89± 0.833	77.76± 1.007	74.56± 0.956
Hepato-pancreas (%)	69.88± 1.821	60.52± 1.619	52.60± 1.398	62.08± 1.538	64.58± 21.28

ANALYSIS OF VARIANCE - MOISTURE

Source	df	SS	MS	F
Tissue	2	3144.689	1572.344	40.107**
M-Stage	4	686.202	171.551	4.376**
Interaction	8	884.918	110.615	2.822
Error	60	2352.200	39.203	-
Total	74	7058.008	95.514	-

** Highly significant

Fig.2 Variations in the concentration of total protein during different maturity stages of P.stylifera

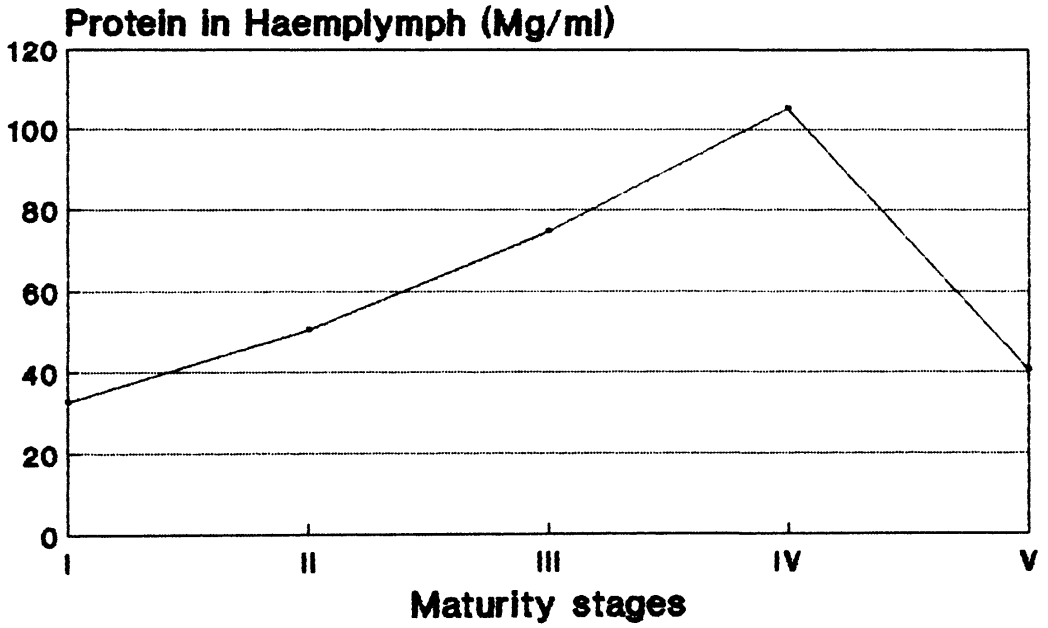


Fig.3 Variation in the concentration of total protein during different maturity stages of P.stylifera

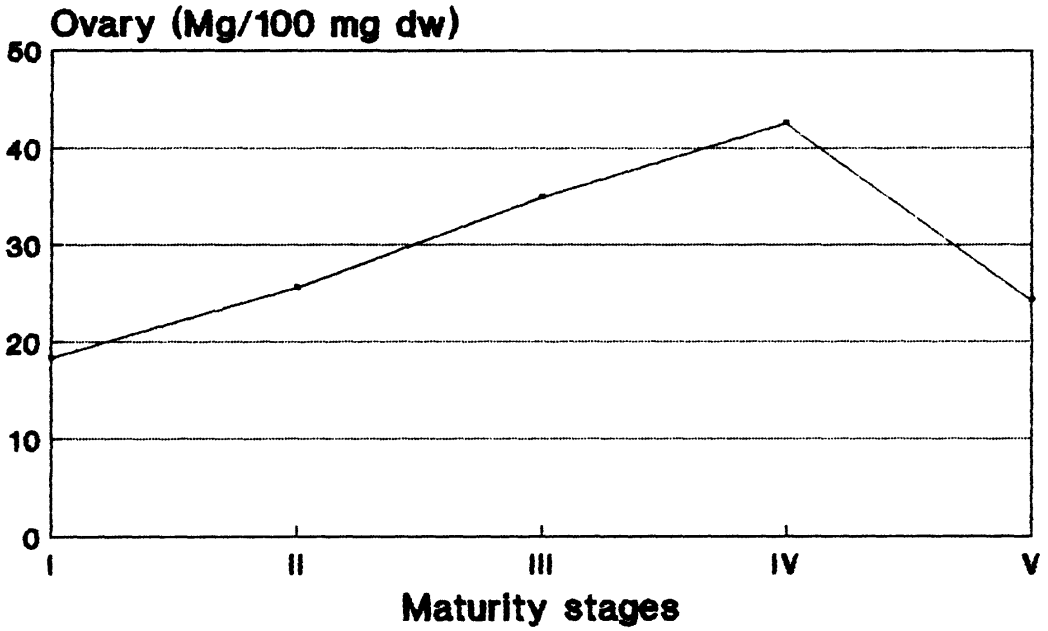


Fig.4 Variation in the concentration of total protein in the muscle in different stages of maturity of P.stylifera

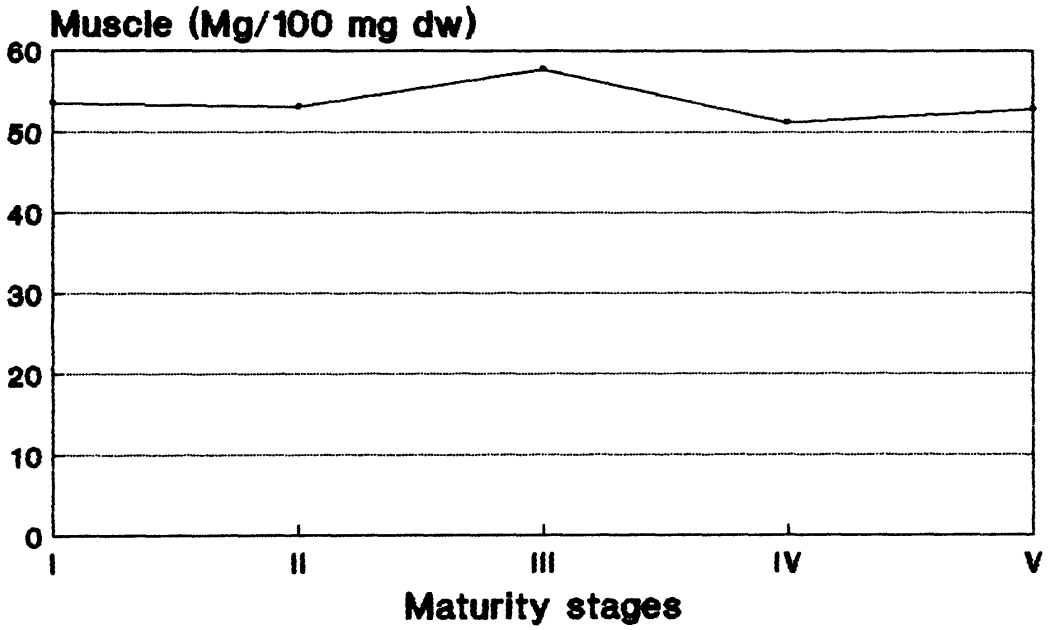


Fig.5 Variations in the concentration of total protein during different maturity stages of P.stylifera

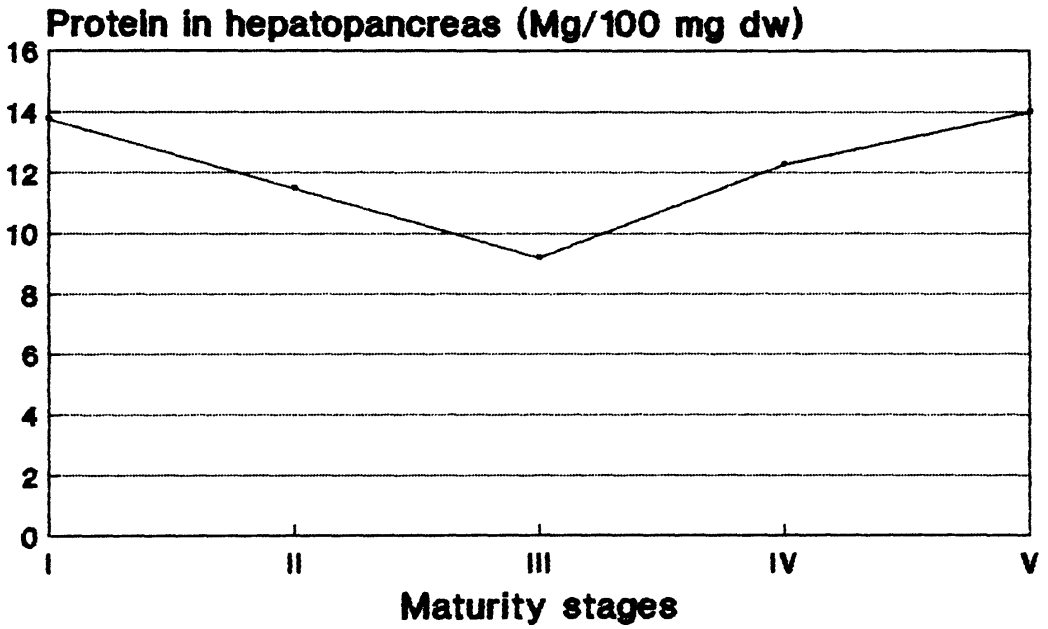


Fig.6 Variations in the concentration of total carbohydrate during different maturity stages of P.stylifera

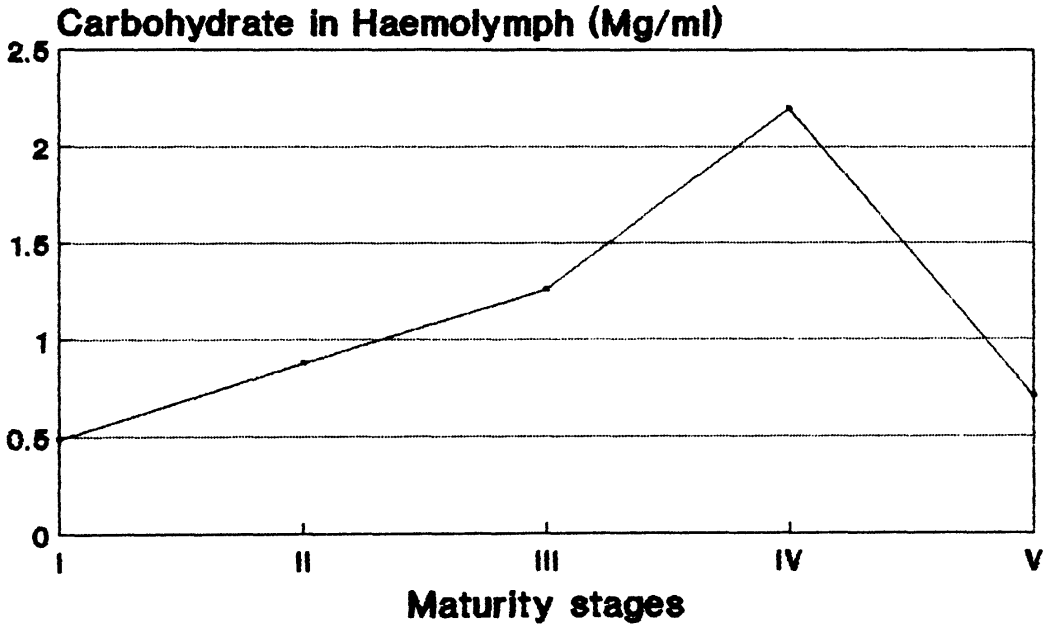


Fig.7 Variations in the concentration of total carbohydrate during different maturity stages of P.stylifera

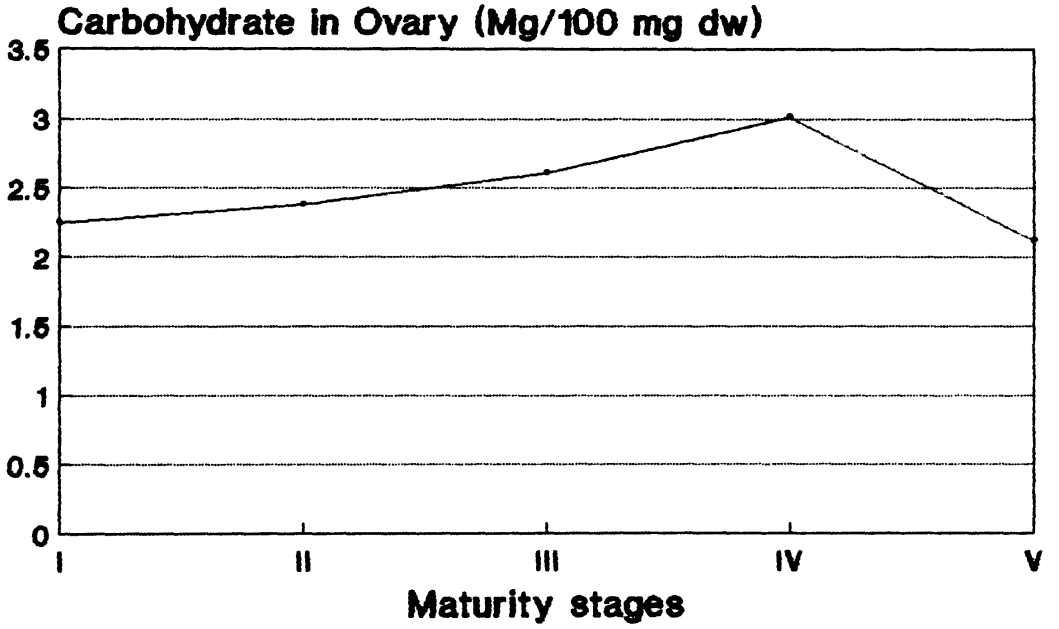


Fig.8 Variations in the concentration of total carbohydrate during different stages of P.stylifera

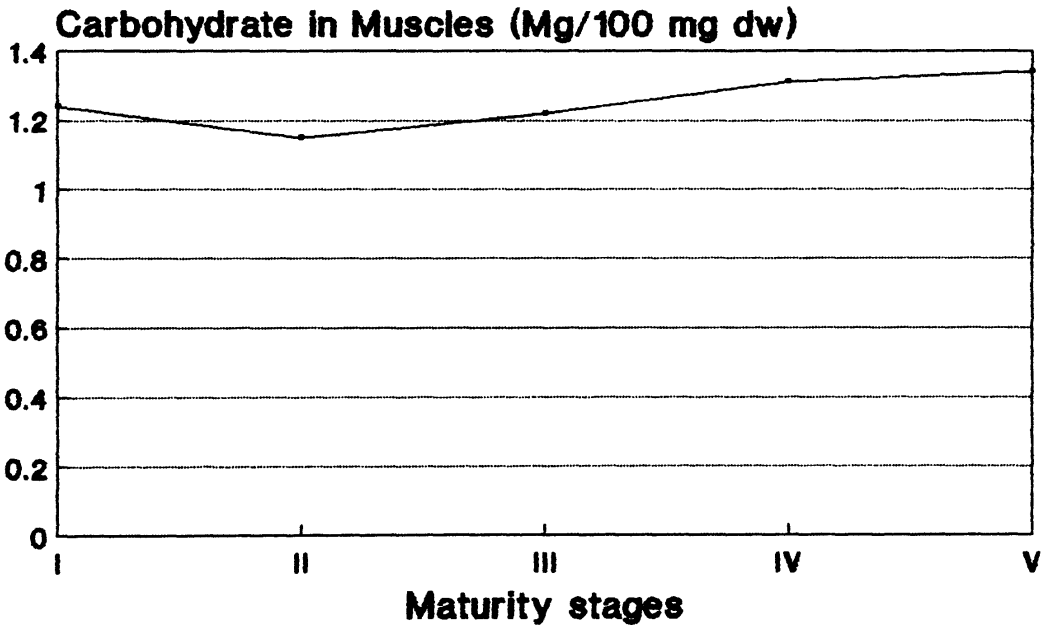


Fig.9 Variations in the concentration of total carbohydrate during different maturity stages of P.stylifera

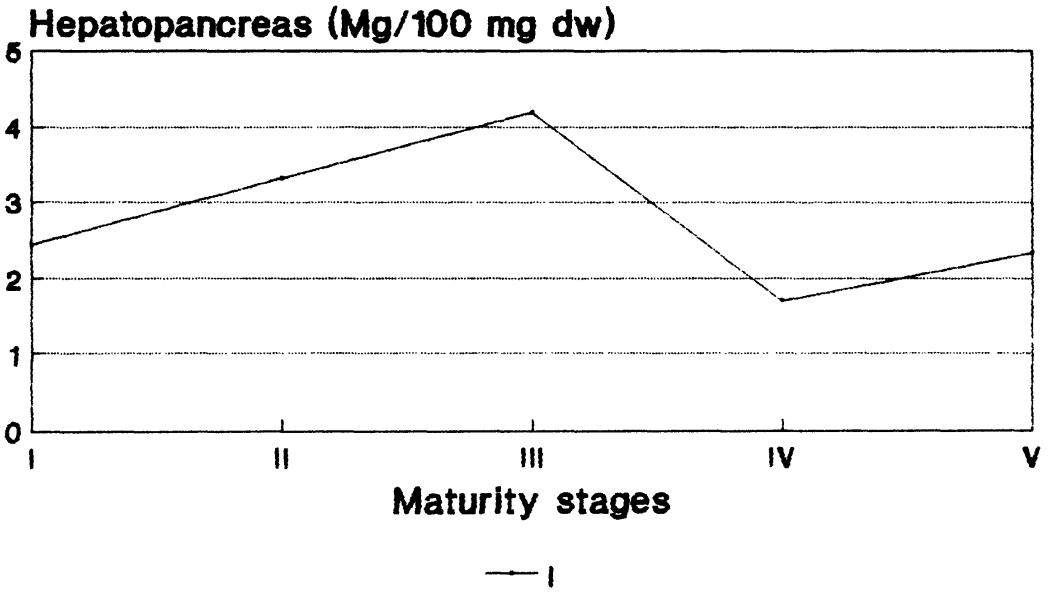


Fig.10 Variations in the concentration of total lipids in different maturity stages of P.stylifera

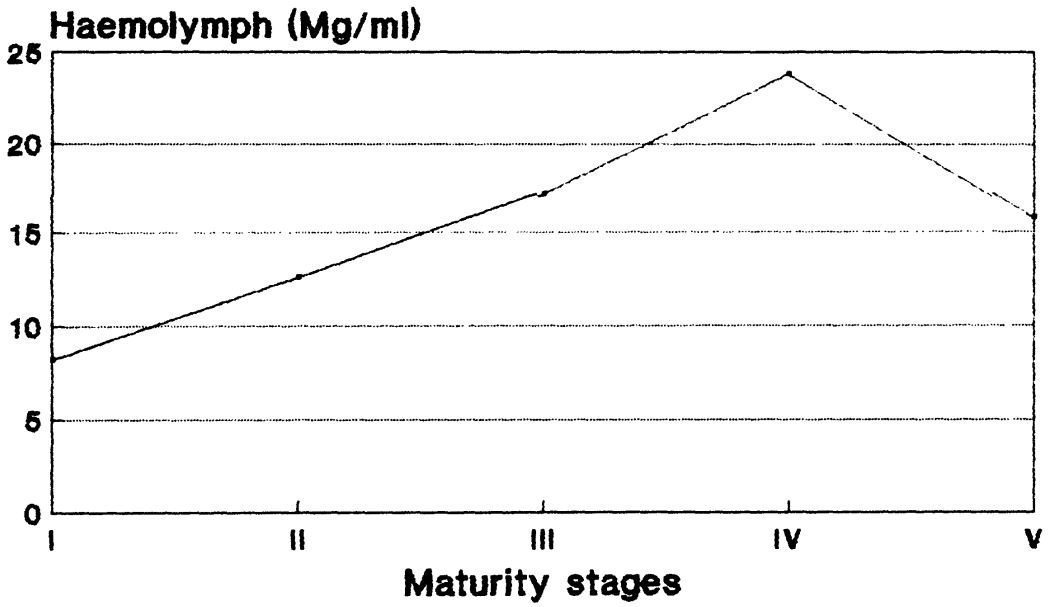


Fig.11 Variation in the concentration of total lipid during different maturity stages of P.stylifera

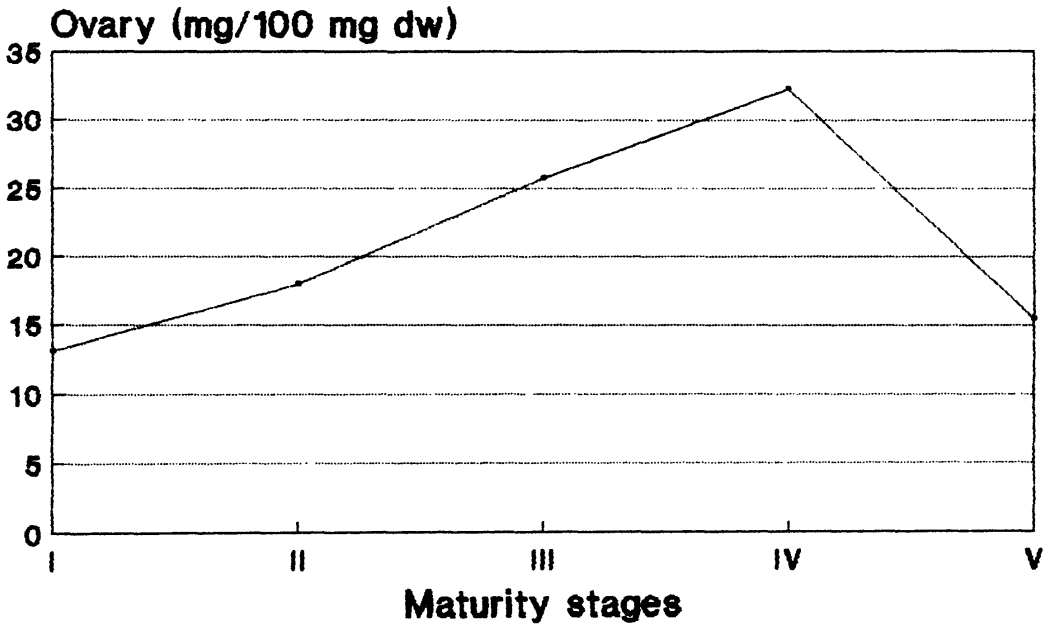


Fig. 12. Variations in the concentration of total lipids during different maturity stages of *P. stylifera*

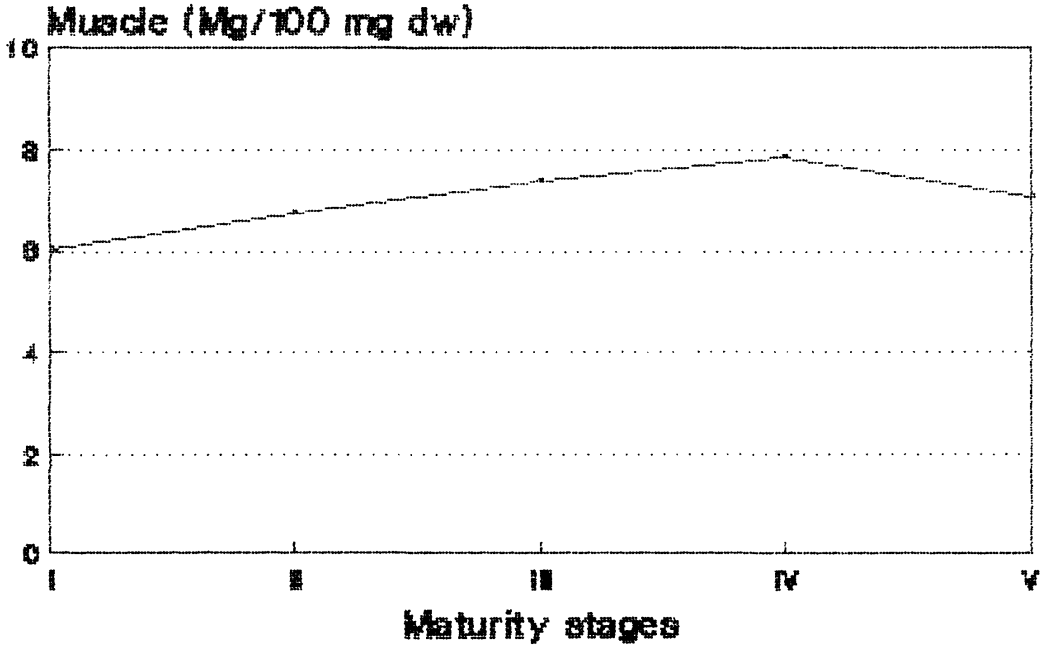


Fig.13 Variation in the concentration of total lipids during different maturity stages of P.stylifera

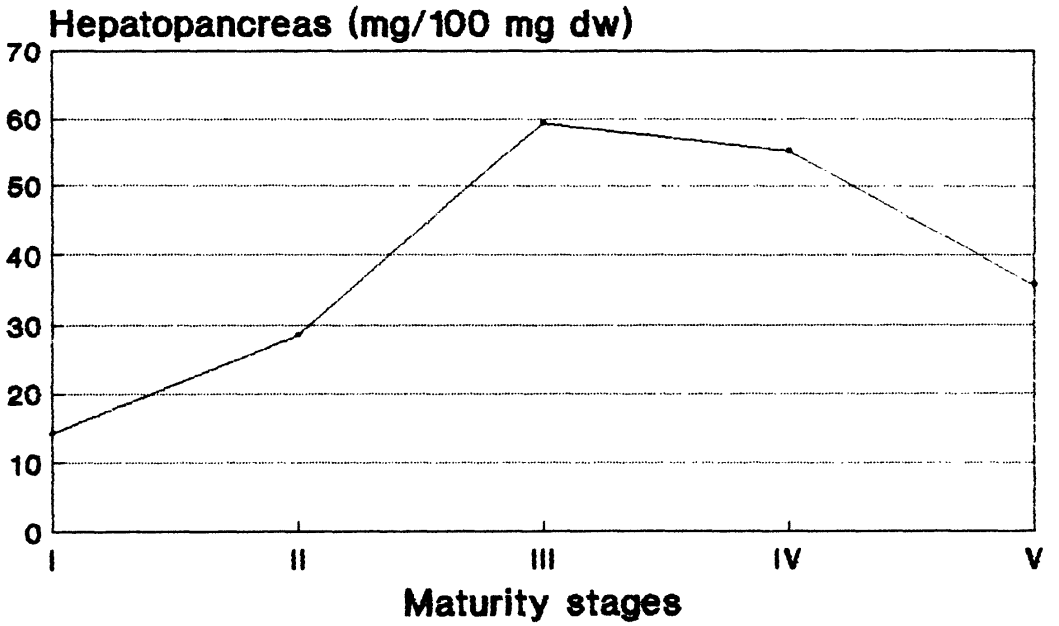


Fig.14 Variations in moisture in ovary during different maturity stages of *P.stylifera*

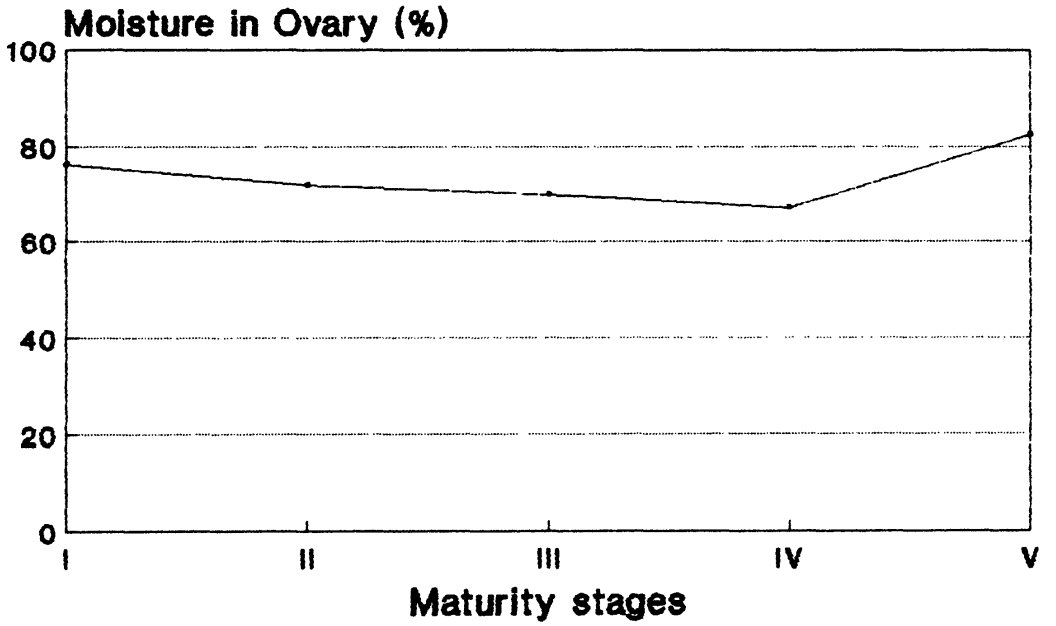


Fig.15 Variation in moisture during different maturity stages of P.stylifera

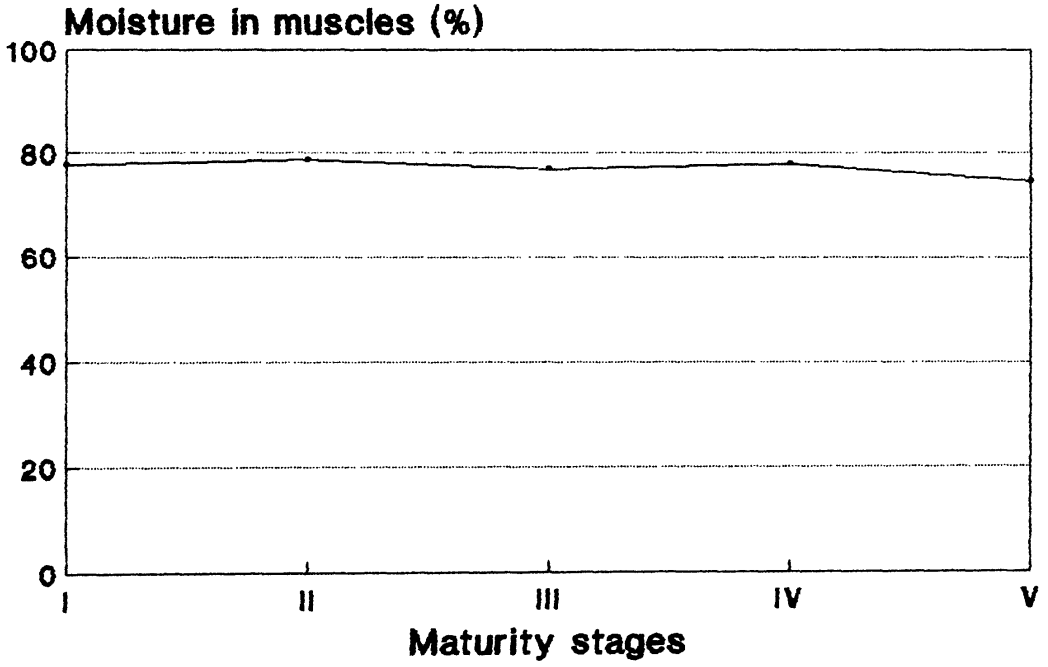
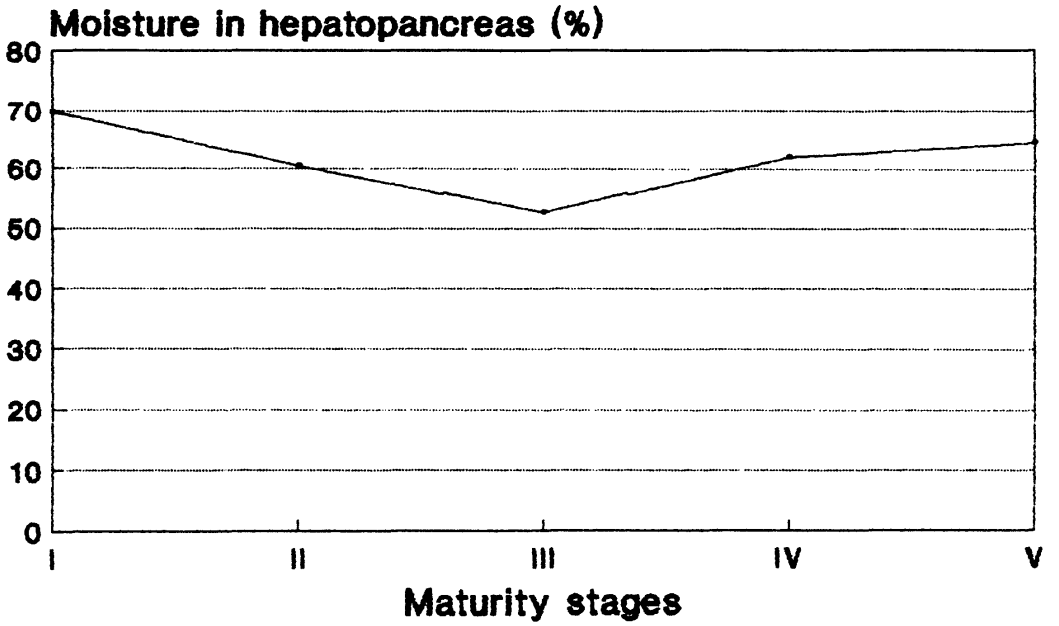


Fig.16 Variations in moisture in hepato-pancreas during different maturity stages of *P.stylifera*



DISCUSSION

Reproduction in shrimp is an energy demanding process. Which includes vitellogenesis, spawning, hatching, followed by subsequent cycles of maturation and spawning. A great amount of energy is channalised to the gonads and this in turn is reflected in the deposition or the depletion of nutrients with the advent or departure of the reproductive period (Lambert and Dehnel, 1974). It is well known that the gonadal maturation, especially maturation of ovary, in the decapod crustaceans involves a cyclical demand for materials and the energy is translocated mainly from the somatic sources (Sastry, 1983). In the foregoing account here, the changes in the major biochemical components in the haemolymph, ovaries, muscle and hepatopancreas have been discussed.

The estimation of various biochemical parameters during the different maturity stages showed the cyclic pattern and accumulation of organic reserves in the haemolymph, ovary and hepatopancreas of the shrimp, P.stylifera. The biochemical composition of muscle tissues did not show any significant variation in relation to the maturation process. In general, these results are in agreement with that observed in other crustaceans by Rahman (1967), Adiyodi (1968), Pillay and Nair (1973), Diwan and Nagabhushanam (1974), Lawrence et al (1979), Shyamasundari and Erribabu (1979), Varadarajan and Subramaniam (1982) and Mohamed and Diwan (1992).

In P.stylifera, during active phases of vitellogenesis, there was a significant rise in the protein levels in the ovary and haemolymph. Peak protein levels were observed in fully mature stage. The increase in protein content of the haemolymph parallel to that observed in the ovary indicated probable plasmal transport of the proteins from the external source as was observed in P.indicus (Mohamed, 1989).

In P.stylifera during active phases of vitellogenesis there was a significant rise in protein level in the ovary as well as in the haemolymph. Peak protein levels were noticed in the fully mature stage. The hike in the protein content of the haemolymph parallel to that observed in the ovary indicated probable plasmal transport of proteins from the external source to the ovary. In the present stage the haemolymph levels varied from 32.72 mg/ml from the immature stage to 105.29 mg/ml in the mature stage, a trend observed also in P.indicus (Mohamed and Diwan, 1992). In other crustaceans, haemolymph protein concentration in general has been reported to be as high as 116 mg/ml in Panulirus longipes (Dall, 1974). In the American lobster H.americansis, Barlow and Ridgeway (1962) reported that the total serum protein was in peak levels during the mature stage due to the mobilization of proteins to the ovary. Accumulation of proteins in the ovary of P.stylifera is commensurate with the established role of proteins in tissue build-up during embryonic development. Similar accumulation of protein in the ovary during the

reproductive cycle has been noted in cirripedes (Barnes *et al*, 1963) in *P.pelagicus*, *M.affinis* and *Uce annulipes* (Pillai and Nair, 1973), in *B.cunicularis* (Diwan and Nagabhushanam, 1974) in the anomuran crab *C.clibanarius* (Varadarajan and Subramoniam, 1982) in *M.dobsoni* (Vasudevappa, 1992) and in *M.idella* (Joshi, 1990).

The results of the present investigation revealed that considerable mobilization of protein takes place from hepatopancreas. Although the content of protein in the hepatopancreas was observed to be poor, there was a progressive decrease in the protein levels during active vitellogenesis in *P.stylifera*. But in the spent stage, the protein content in the hepatopancreas was found to be restored to its original level indicating considerable mobilization of protein during vitellogenesis. Similar trends in protein content in hepatopancreas have been reported in *P.monodon* by Dy Penaflorida (1990), in *P.indicus* by Mohamed and Diwan (1992) and in *M.dobsoni* by Vasudevappa (1992). Adiyodi (1969) reported that in *Paratelphusa hydrodromous* the hepatopancreas was not the major source of vitellogenic proteins but will be the source of vitellogenic precursor. However, in *P.stylifera*, the present study indicated some degree of transportation of proteins to the ovary through the haemolymph, and additional evidence to the statement comes from the observation of low initial concentration of protein found in ovary during stage I (18.34%). Conversely in *C.clibanarius* Varadarajan and Subramoniam (1982) found high

initial concentration of protein (22%) in the ovary obtained through autosynthetic means which, therefore, precludes the need for proteins through and external source. In P.stylifera, apparantly, autosynthetic proteins alone are insufficient for yolk formation and therefore heterosynthetic proteins derived from the hepatopancreas are also probably incorporated into the yolk.

Muscle protein content in the present study did not show significant variations but without any definite trend in relation to the stages of maturation and therefore this could not be correlated to the process of vitellogenesis. Mohamed and Diwan (1992) also did not find any trend in muscle protein levels during maturation in P.indicus. In M.dobsoni, Vasudevappa (1992) observed that the variation in the muscle protein was not statistically significant but in this case low levels of protein in immature and spent females and relatively higher levels in early maturing to mature females, indicated the increase in muscle protein during vitellogenesis. Claybrook (1983) reported that muscle proteins are mainly involved in process of tissue growth and metabolism in crustaceans. Contrary to the present observation in P.stylifera, Achuthankutty and Parulekar (1984) reported higher levels of protein in young shrimps than in sexually mature specimens of M.affinis, M.dobsoni, P.merguiensis and P.stylifera.

In the haemolymph of P.stylifera, the carbohydrate content increased fourfold during stage IV but decreased drastically in

spent females. Similar trend was observed in P.indicus (Mohamed and Diwan, 1992). Vasudevappa (1992) reported a threefold increase in carbohydrate content of haemolymph during maturation. In M.idella, the carbohydrate level increased with advancement of maturation and the highest value was obtained in fourth stage (Joshi 1990). A doubling of the haemolymph glucose level was observed by Dean and Vernberg (1965) in Callinectes sapidus and by Telford (1968) in Cancer borealis as the crab became ovigerous. In P.hardwickii, Nagabhushanam and Kulkarni (1980) reported a continuous increase in haemolymph level on the ovarian development proceeded from stage I to IV and then a sudden decrease in the levels was observed in stage V. Adiyodi and Adiyodi (1970 b) reported that in P.hydrodromous, the sugars present in the hepatopancreas and haemolymph were found in some abundance in the ovary during early stages of vitellogenesis but disappear progressively as the proteins in the ovary became conjugated in the course of yolk formation. It was suggested by Nagabhushanam et al (1980) that the sugar present in the haemolymph and hepatopancreas may be utilized mainly for the construction of yolk proteins and perhaps also as a fuel during the active vitellogenic process. The increasing trend of carbohydrate content observed in P.stylifera with the advancement of maturity stages also seemed to indicate transport of carbohydrate substances to the ovary for identical reason.

In the ovary of P.stylifera, the carbohydrate content remained more or less static with a small peak in the stage IV.

Similar trend was noted in P.indicus Mohamed and Diwan, (1992). In C.clibanarus, Varadarajan and Subramoniam (1982) observed that the carbohydrate levels in the ovary decreased to very low levels during the ripe stage. They suggested the possibility of carbohydrate levels in the ovary being constant but the relative amount of other macromolecules reducing its percentage in later stages.

In the hepatopancreases of ripe females a sharp decline in the carbohydrate content was observed in the present study indicating the possible transport of carbohydrate substances from the storage organ to the ovary. Similar observations were made in P.indicus (Mohamed and Diwan, 1992). Identical conclusions were drawn by Adiyodi and Adiyodi (1970 b) when they found that free sugars like glucose, galactose and sucrose in the hepatopancreas of P.hydrodromous underwent quantitative and qualitative cyclic fluctuation related to the ovarian cycle. Trujillo and Luna (1981) have clearly demonstrated the mobilization of glycogen from hepatopancreas to gonads during ovarian development in P.notalis. A similar pattern of variation in carbohydrate was observed in M.dobsoni with relatively higher levels in early vitellogenic, late vitellogenic and vitellogenic stages (Vasudevappa, 1992).

In several decapod crustaceans, the haemolymph lipid exists as a complex lipoprotein moeity (Chang and O'Connor, 1983). In P.stylifera, the changes in the levels of different biochemical

components are reflected in the haemolymph as observed in P.indicus (Mohamed and Diwan, 1992). Thus, the haemolymph lipid levels rose to a peak in stage IV and subsequently decreased slightly in spent females indicating transport of lipidic material to the ovary and its utilization in the maturation process. Similar variations in the haemolymph lipid content in relation to reproductive cycle was observed by Teshima and Kanazawa (1978) in P.japonicus. In spent recovering P.stylifera, the lipid content remained at comparatively high levels due to the possible resorption of nutrient relict oocytes, which involves retransport of nutrient material from the ovary back to the storage site. Adiyodi (1985) attributed the protein levels of haemolymph of crustaceans during spent stages to be due to the proteins from resorbing oocytes. The high haemolymph lipid levels in spent P.stylifera may be attributed to the rapid rematuration capabilities of penaeid shrimps reported by Mohamed et al (1981). The comparatively high levels of lipids present in the hepatopancreas of spent females lend additional support for this view. Asokan (1984) in P.indicus, Castille and Lawrence (1989) in P.aztecus and P.setiferus reported the gradual build up in ovarian lipid concentration during maturation and a sharp decline in spent ovaries. In the present study, on P.stylifera, an inverse relationship was observed between ovarian and hepatic lipid content similar to the observations made in P.indicus by Mohamed and Diwan (1992). In P.stylifera, as the content of ovarian lipids increased, there was a concurrent decrease in the

lipid levels in the hepatopancreas. The hepatopancreas has been identified as the principal storage site for lipids in crustaceans (Chang and O'Connor, 1983). Apparently, large quantities of this stored lipid is mobilized to the ovary in P.stylifera during active vitellogenesis through the haemolymph. In the pink shrimp, P.duorarum, Gehring (1974) followed the changes in total lipids, natural lipids, phospholipids, triglycerides and sterols in the ovary with maturation and found peak levels in the late maturing stage. In P.japonicus, Guary et al (1975) reported that the increasing trend of various fatty acids was related to ovarian maturation. Similar variations in the lipid composition during ovarian maturation was reported by Middleditch et al (1980) in P.setiferus and Teshima and Kanazawa (1983) in P.japonicus, Shaikhahmud and Magar (1957) recorded higher lipid content in various body tissues of female P.stylifera. Galois (1984) observed large accumulation of phospholipid and triglycerides in the ovary of P.indicus during vitellogenesis, but discounted the role of hepatopancreas in supplying these to the ovary. He suggested that direct input of lipids from feeding to be the main source. However, the trend in variation of hepatopancreatic lipid in conjunction with maturation observed in the present study seems to suggest that the hepatopancreas is the major source of ovarian lipids. Similar conclusions were drawn by Gilbert and O'Connor (1970) in their review on lipid metabolism in arthopods and Varadarajan and Subramoniam (1982) in the anomuran, C.clibanarius.

Moisture, the major component of the wet body tissue showed significant variations. The water content showed a declining trend both in the ovary and hepatopancreas during vitellogenesis. Similar trend was observed in P.indicus Mohamed & Diwan, 1992) and M.dobsoni (Vasudevappa, 1992). In an earlier study on P.indicus from South African coast, Read and Caulton (1980) observed a decrease in fresh mass, in spite of the increase in ovarian mass due to a loss of water during ovarian maturation. Joshi (1990) observed a decrease in hepatic moisture content from first stage to third stage of maturation in M.idella, indicating the building up of reserves in the hepatopancreas and thereafter increase upto the sixth stage indicating utilization of the reserves. George and Patel (1956) observed an inverse relationship between water and fat content of the gonad in the Bombay lobster. This increase in fat content was evidently associated with gonadal development. In M.affinis and P.pelagicus, Pillay and Nair (1973) also observed an inverse relationship between water content and gonadal development. In general, the water content in the ovary tended to decline along with an increase in total mass of organic substances in the ovary. Considerable variation in the water content takes place during the moult cycle in crustaceans and control of water in tissues has been identified as a possible mechanism by means of which the animal achieves tissue growth (Yamaoka and Scheer, 1978). Similarly, it is probable that the water is lost in ovarian and hepatic tissues due to the continuous deposition of

organic materials resulting in an increase in dry weight of the body.

In crustaceans, yolk is the nutritive material accumulated in substantial quantities in the ooplasm to meet the basic requirements of embryonic development, and moreover the composition of yolk varies from species to species (Adiyodi & Subramoniam, 1983). The biochemical studies on the mature ovary of P.stylifera revealed that water formed the most significant portion of the yolk and proteins and lipids constituted the major organic reserves. It is generally accepted that the yolk proteins provide the basic structural material needed for tissue build up during embryonic development, while lipids serve as major fuel (Adiyodi & Subramoniam, 1983). Evidence derived from the present study indicated that a significant portion of the proteins and carbohydrates and almost all the lipids found in the yolk of P.stylifera are probably derived from the hepatopancreas via the haemolymph through heterosynthesis.

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SUMMARY

1. The metabolic component like protein, carbohydrate, lipid and moisture in the haemolymph, ovary, muscle and hepatopancreas of female P.stylifera during the five maturity stages were estimated.

2. Significant variations in the biochemical parameters in relation to the maturity stages were observed in the haemolymph, ovary and hepatopancreas. The biochemical composition of muscle tissues did not show any significant variation in relation to maturation.

3. The changes in the levels of different biochemical parameters in the ovary and hepatopancreas were always reflected in the haemolymph where the content of proteins carbohydrates and lipids increased steadily from very low levels prevailing in stage I to peak levels in stage IV. In spent recovering females (Stage V), there was a significant drop in the levels of all these biochemical parameters.

4. The content of protein, carbohydrate, and lipid in the ovary gradually increased from stage I to maximum levels in stage IV (fully ripe). A significant decrease in the levels of all these biochemical components was observed in the ovaries of stage V (spent recovering) females. In contrast, the levels of moisture were observed to decrease

from an initial high concentration in stage I to very low concentration in stage IV. Subsequently, the levels of moisture content registered an increase in stage V. Maximum moisture content was observed in the ovaries of stage V (spent recovering females).

5. The carbohydrate and lipid, content in hepatopancreas, gradually increased from low level in stage I maximum levels in stage III and thereafter there was a gradual decrease. Maximum content of hepatic proteins was observed in stage I and V and minimum in stage III. The moisture content in the hepatopancreas also showed a similar trend.

6. The contents of proteins, carbohydrates, lipids and moisture in the muscle tissues were found to vary during the different maturity stages without a definite trend or pattern.

7. Moisture was found to be the main component of the fully formed yolk. Proteins and lipids formed the next major components, while the carbohydrates were found only in small quantities in the fully formed yolk in P.stylifera.

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