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Biochemical Changes during Gonadal Maturation in *Portunus pelagicus* (Linnaeus, 1758)

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The changes in moisture, protein, lipid, carbohydrate and carotenoids in the ovary, hepatopancreas, muscle and haemolymph during the process of gonadal maturation of the crab *Portunus pelagicus* (Linnaeus, 1758) were investigated. There was significant difference ($p<0.01$) in moisture, protein and carotenoid levels at various maturity stages. The lipid content showed significant difference ($p<0.01$) in all the tissues except in muscle tissue and the carbohydrate values showed significant difference ($p<0.01$) only for ovary and hepatopancreas. The moisture content decreased with gonadal maturation in all the tissues. The protein titre increased with maturity in ovary, muscle and haemolymph whereas in hepatopancreas it registered a decrease. The lipid values of all the tissues and the haemolymph showed an increasing trend with maturation. The carbohydrate level also increased with gonadal maturity in the tissues and haemolymph. The carotenoid content followed an increasing trend till mature stage in the ovary whereas in the hepatopancreas and haemolymph it increased till the late maturing stage.

Keywords: ***Portunus pelagicus*, biochemical constituents, gonadal maturation, vitellogenesis**

Marine blue swimmer crab *Portunus pelagicus* (Linnaeus, 1758) is one of the candidate species for culture due to its high growth rate, attractive colour and export value. In order to popularise its culture in the country and also to aid conservation, attempts are being made to standardize the hatchery technology for this species. A study on the dynamics of lipids, proteins, carbohydrates and carotenoid pigments in ovary, hepatopancreas, muscle and haemolymph during the process of ovarian maturation is an effective approach towards increasing our knowledge regarding crustacean broodstock nutrition (Harrison, 1990). In crustaceans, a great amount of energy gets channeled to the gonads during reproduction, which is reflected in the deposition or depletion of nutrients with the advent or departure of the reproductive period (Lambert & Dehnel, 1974). Moisture, proteins, lipids, carbohydrates and carotenoids are the major biochemical constituents that undergo variation during gonadal maturation. In a study on the water content in the hepatopancreas, muscle and ovary of *Clibanarius longitarsus*, Khan *et al.* (1977) observed an inverse relation between moisture content and protein and lipid values. Kulkarni & Nagabhushanam (1979) observed a decrease in the protein titre of hepatopancreas with its increment in the ovary during maturation in *Parapenaeopsis hardwickii.* A significant increase in protein and lipid content of the ovary in the late maturing and mature stages at the expense of carbohydrate was recorded in *Uca tangeri* by Mourente *et al.* (1994). According to O' Connor & Gilbert (1968), glycogen and lipids, the predominant organic reserves of many crustaceans get accumulated in the midgut gland and ovaries during gonadal development. It was noted that the reserves stored in the hepatopancreas get mobilized to the ovaries immediately before and during vitellogenesis in the brachyuran crabs (Kyomo, 1988). Lipids stored in hepatopancreas are transported to ovaries during vitellogenesis in *Farfantepenaeus aztecus* and *Litopenaeus setiferus* (Castille & Lawrence, 1989). Adiyodi (1968)

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suggested that lipids are carried by lipoproteins to the ovary during vitellogenesis in crustaceans. During early maturation, free and esterified carotenoids accumulate in the hepatopancreas and during late maturation they are mobilized *via* haemolymhph to ovaries. This accumulation of carotenoids in the ovaries during maturation results in their darkening, on which the staging of females into different maturation classes is based (Harrison, 1990). Though some aspects of the biochemical composition of certain tissues of *P. pelagicus* have been investigated by earlier workers like Pillay & Nair (1973), Hamsa (1978) and Siddiqui *et al.* (1987), a comprehensive study of all the major constituents in various tissues and haemolymph at all the maturity stages is yet to be carried out. The present study was aimed at understanding the variation in biochemical constituents such as moisture, protein, carbohydrate, lipid and carotenoid in the ovary, hepatopancreas, muscle and haemolymph of *P. pelagicus* at different reproductive stages such as immature, early maturing, late maturing, mature and spent.

Materials and Methods

Live specimens of female *P. pelagicus* were collected monthly from gillnetters operating in the Gulf of Mannar, off Mandapam (9^0 11′ N, 78^0 56′ E) in Tamil Nadu during October-November 2006. The animals were carefully transported to the laboratory and kept in 1 tonne Fibre glass reinforced plastic (FRP) tanks with continuous aeration. After a conditioning period of one week, healthy specimens were segregated and measured across the carapace between the tips of the ninth antero-lateral spines to the nearest 1 mm and the total body weight to the nearest 1.0 g. The animals of size ranging from 130 to 150 mm were selected for the study. Feeding was done with raw squid and clam meat (1:1) *ad libitum* daily. Reproductive stages were assigned based on macroscopic characteristics described for *P. sanguinolentus* by Ryan (1967) as immature (IM), early maturing (EM), late maturing (LM), mature (M) and spent (SP). Haemolymph samples drawn from the heart using hypodermic syringe with No. 22 needle after rinsing with modified citrate EDTA buffer (Soderhall & Smith, 1983) and the tissue samples of ovary, hepatopancreas and muscle, excised from live crabs at different reproductive stages were stored at -20°C until analysis. Each of the estimations was repeated thrice to minimize error. For estimating the moisture content as per Horwitz (1970), animals were dissected live and known weights of samples of ovary, hepatopancreas and muscle tissues collected from each reproductive stage were kept in hot air oven at 102°C and dried to a constant weight. The total soluble protein was estimated as per the Folin-Ciocalteu method of Lowry *et al.* (1951) with bovine serum albumin (BSA) as standard. For this, 25 mg each of the wet tissues of ovary, hepatopancreas and muscle and 0.1 ml of the haemolymph (suspension) from each reproductive stage were taken in clean dry test tubes, and to that 1 ml of 10% trichloro acetic acid (TCA) was added. The mixtures were homogenized in a tissue homogenizer and centrifuged at 3000 rpm for 15 minutes. The protein precipitate in each tube was dissolved in 5 ml of 1 N NaOH and the standard methodology was followed. The supernatant obtained was used for the estimation of carbohydrate content following Dubois *et al.* (1956). The lipids were quantitatively determined by Sulpho-phosphovanillin method of Barnes & Blackstock (1973). For this, 10 mg each of the wet tissues of ovary, hepatopancreas and muscle and 0.1 ml of the haemolymph from each reproductive stage taken in clean dry test tubes were homogenized in chloroform:methanol mixture (2:1, v/v CH_3Cl:CH_3OH) and kept overnight in tightly stoppered test tubes at 4°C for complete extraction. The contents were mixed well and centrifuged at 3000 rpm for 15 minutes. The supernatant containing the lipids was transformed to clean, dry test tubes. From this lipid extract, 0.5 ml each was taken in a separate test tube and dried in vacuum over silica gel in a desiccator. To the dried samples, 0.5 ml of concentrated

sulphuric acid was added and shaken well. The tubes were then plugged with non-absorbant cotton wool, heated at 100°C in a boiling water bath for 10 minutes and rapidly cooled to room temperature under running tap water. To 0.1 ml of this acid digest, 2.5 ml of phosphovanillin reagent was added and mixed well in a cyclomixer. The pink-red colour developed was read after 30 minutes at 520 nm in UV-spectrophotometer (Genesys) along with the standard cholesterol (8 mg cholesterol in 10 ml 2:1 v/v CH_3Cl:CH_3OH mixture) and reagent blanks. (Phosphovanillin reagent was prepared by mixing 800 ml orthophosphoric acid, 200 ml double distilled water and 2 g vanillin). For estimating carotenoid content, 1 g each of wet tissues of ovary and hepatopancreas and 1 ml of haemolymph were collected from different reproductive stages and the analysis was carried out immediately following Olson (1979).

Results and Discussion

The moisture content of the ovary and hepatopancreas showed a gradual decrease till mature stage and a sharp increase towards the spent stage (Fig. 1). The maximum and minimum values of moisture content in the ovary and hepatopancreas were at the spent and mature stages, respectively. In the muscle tissue, though the pattern was somewhat similar to that of ovary and hepatopancreas, the minimum value was recorded in the late maturing stage followed by an increase in the titre. The variation in moisture content in all the tissues in the present study was statistically significant ($p<0.01$). The moisture content in the ovary and muscle tissue followed an inverse relationship with respect to protein and lipid values. This observation agrees to the findings by Diwan & Mohamed (2007) in *Fenneropenaeus indicus*, Vasudevappa (1992) in *Metapenaeus dobsoni* and Mohammad *et al.* (2004) in *Scylla serrata*. Khan *et al.* (1977) reported a decrease in moisture in muscle and hepatopancreas, with maturity in *Clibanarius longitarsus*. According to Diwan & Mohamed (2007) the continuous deposition of organic materials in ovarian and hepatic tissues at the time of maturation results in considerable loss of water.

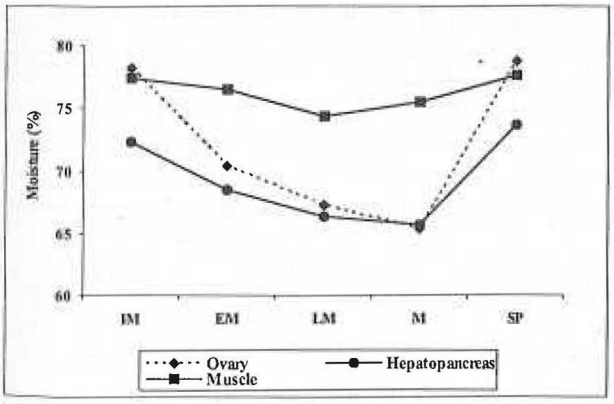

Fig. 1. Variation in moisture content in the tissues of ovary, hepatopancreas and muscle during gonadal maturation in *Portunus pelagicus* (IM-immature, EM-early maturing, LM-late maturing, M-mature, SP-spent).

The protein values in the tissue samples and haemolymph of *P. pelagicus* at different maturity stages showed significant difference ($p<0.01$). In the ovary, the protein value increased gradually with gonadal maturation and reached a maximum at the mature stage and decreased thereafter (Fig. 2). This observation is in agreement with the reports of Varadarajan & Subramoniam (1982) in *C. clibanarius* and Vijayakumaran & Radhakrishnan (2002) in *Panulirus homarus*. According to Adiyodi & Subramoniam (1983) this steady increase can be attributed to the combined action of both autosynthesis and heterosynthesis at the beginning and towards the end of vitellogenesis, respectively. Though the protein level of the ovary dropped after the mature stage, its level at the spent stage was higher than that at the immature stage. The marginal increment shown by the spent ovary in comparison to the immature ovary could be attributed to the presence of residual oocytes. In the immature ovary, even if vitellogenesis would have commenced, it appears to have occurred rather slowly as evidenced by the lowest protein value as opined by Adiyodi & Subramoniam (1983). In the hepatopan-

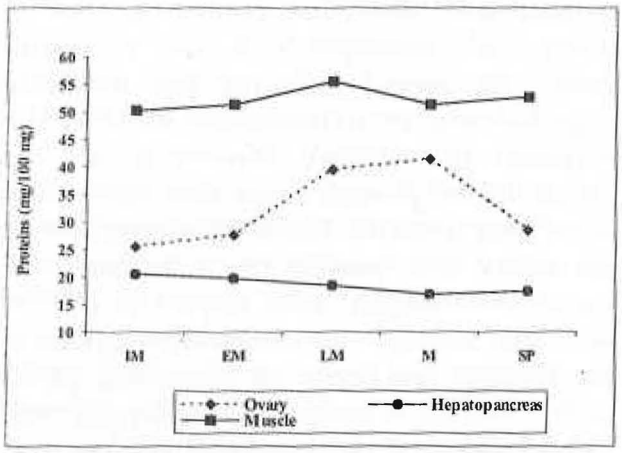

Fig. 2. Variation in protein content in the tissues of ovary, hepatopancreas and muscle during gonadal maturation in *Portunus pelagicus* (IM-immature, EM-early maturing, LM-late maturing, M-mature, SP-spent).

creas the protein values showed a decreasing trend with gonadal maturity. Contrary to that in the ovary, in hepatopancreas the maximum value was observed at the immature stage and the minimum at the mature stage. Similar observations showing a negative correlation between the protein contents in the ovary and in the hepatopancreas were made by Diwan & Nagabhushanam (1974) in *Barytelphusa cunicularis* and Nagabhushanam & Kulkarni (1977) in *Emerita holthuisi*. This inverse relationship is due to mobilization of protein from the hepatopancreas to the ovary (Khan *et al.*, 1977). The protein content of hepatopancreas reduced gradually with vitellogenesis, which probably indicated the priority for autosynthesis to heterosynthesis (Adiyodi & Subramoniam 1983). Though significant difference ($p<0.01$) was observed in the muscle protein values at different maturity stages it did not seem to follow any particular pattern. This could be due to the involvement of muscle proteins in growth and metabolism rather than in reproduction as suggested by Claybrook (1983). The maximum and minimum values recorded by muscle protein were at late maturing and immature stages, respectively. The protein content of the haemolymph followed a pattern similar to that of the ovary, which increased with vitellogenesis (Table 1). The simultaneous increase of haemolymph and ovary protein contents evidenced the role of haemolymph as a carrier of protein from other tissues to ovary during vitellogenesis (Diwan & Mohammed, 2007).

Among the tissues investigated, the hepatopancreas showed the maximum lipid content at all the maturity stages (Fig 3). The lipid values of hepatopancreas showed significant difference ($p<0.01$) between different maturity stages and followed an increasing trend with gonadal maturity, reaching a maximum at the mature stage.

Table 1. Variation in biochemical constituents in haemolymph during gonadal maturation in *Portunus pelagicus*

Biochemical constituents	Gonadal maturity stages				
	Immature	Early maturing	Late maturing	Mature	Spent
Protein (mg/ml of haemolymph)	47.29±0.564	54.64±0.880	85.24±0.700	88.52±0.711	39.70±0.745
Lipids (mg/ml of haemolymph)	12.35±0.370	14.25±0.694	23.37±0.764	22.69±0.512	13.71±0.942
Carbohydrates (mg/ml of haemolymph)	0.47±0.207	0.66±0.210	0.94±0.216	1.43±0.304	0.76±0.310
Carotenoids (µg/ml of haemolymph)	12.17±0.325	16.43±0.296	28.52±0.365	26.40±0.373	21.24±0.662

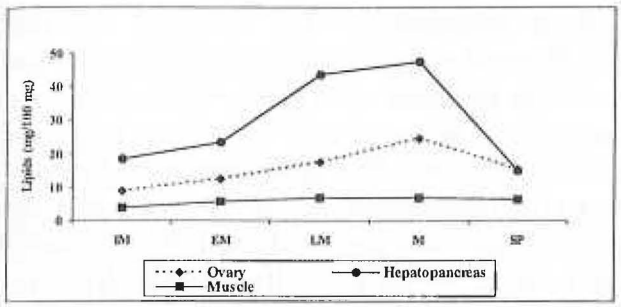

Fig. 3. Variation in lipid content in the tissues of ovary, hepatopancreas and muscle during gonadal maturation in *Portunus pelagicus* (IM-immature, EM-early maturing, LM-late maturing, M-mature, SP-spent)

Increase in the hepatopancreatic lipid content with maturation has been reported by Millamena & Pascal (1990) in *Penaeus monodon* and Vasudevappa (1992) in *M. dobsoni*. The lipid values in the ovary at different maturity stages also showed significant difference ($p<0.01$). The values increased with vitellogenesis recording a maximum at the 'mature' stage. Similar observations were made by Adiyodi & Adiyodi (1970) and Wouters *et al.* (2001) in *Paratelphusa hydrodromous* and *Litopenaeus vannamei*, respectively. This is probably due to the sequestration of lipid globules from outside the oocyte, which occurred during the late maturing stage. Though the lipid content in muscle of *P. pelagicus* showed difference at different maturity stages it was not significant. A similar observation was made by Vijayakumaran & Radhakrishnan (2002) in *P. homarus*, where the lipid content gradually increased till mature stage and then decreased slightly. Diwan & Mohamed (2007) in *F. indicus* and Vasudevappa (1992) in *M. dobsoni* also recorded a similar pattern, but with a pronounced slope towards the spent stage. The haemolymph lipid value increased steadily till the late maturing stage and decreased thereafter (Table 1). Yano & Chinzei (1987), Fainzilber *et al.* (1992) and Diwan & Mohamed (2007) indicated the role of haemolymph in lipid transportation during vitellogenesis in penaeid shrimps.

The carbohydrate content in the ovary was found to differ significantly ($p<0.01$) at different maturity stages and recorded a steady increase with gonadal maturation (Fig 4). This observation is in agreement with the reports of Vasudevappa (1992) in *M. dobsoni* and Bose (1995) in *P. semisulcatus*. A significant difference ($P<0.01$) in carbohydrate values was shown in the haemolymph also, which increased almost three-fold with gonadal maturation (Table 1). This is similar to the observations by Bose (1995) in *P. semisulcatus* and by Diwan & Mohamed (2007) in *F. indicus*. Though the hepatopancreatic and muscle carbohydrate values followed an increasing trend with vitellogenesis, the differences were not significant. Comparable results were obtained by Khan *et al.* (1977) in *C. longitarsus* and Millamena & Pascal (1990) in *P. monodon*. So it may be possible that there is only a little mobilization of carbohydrates from hepatopancreas and muscle to ovary during vitellogenesis.

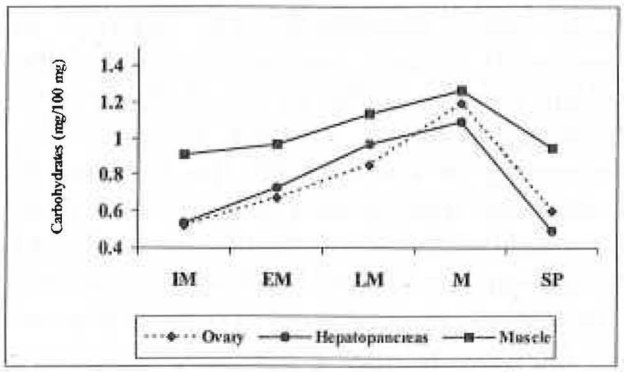

Fig. 4. Variation in carbohydrate content in the tissues of ovary, hepatopancreas and muscle during gonadal maturation in *Portunus pelagicus* (IM-immature, EM-early maturing, LM-late maturing, M-mature, SP-spent)

Significant difference ($p<0.01$) was observed in the carotenoid content at different stages of maturity in the ovary, hepatopancreas and haemolymph of *P. pelagicus*. The carotenoids were recorded maximum in the hepatopancreas at all the maturity stages (Fig 5). It is possible that the hepatopancreas has acted as a storehouse for carotenoids registering a maximum and minimum at the late maturing and immature stages, respectively. Castillo *et al.* (1982) observed

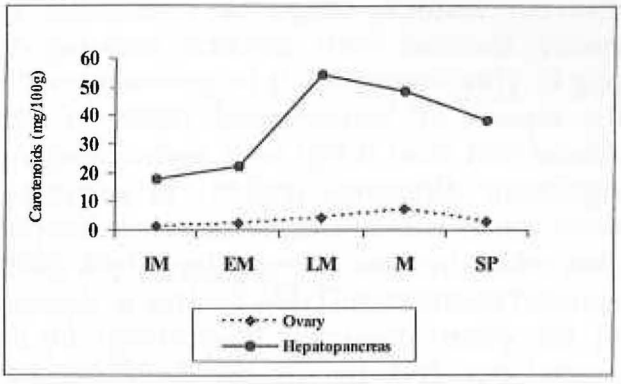

Fig. 5. Variation in carotenoids in the tissues of ovary and hepatopancreas during gonadal maturation in *Portunus pelagicus* (IM-immature, EM-early maturing, LM-late maturing, M-mature, SP-spent)

hepatopancreas to have the highest value for carotenoids among the tissues analysed. The carotenoid content in the ovary showed a gradual increase with maturation. These findings agree with the reports of Vasudevappa (1992), Bose (1995) and Cabello *et al.* (2003) in *M. dobsoni, P. semisulcatus* and *L. vannamei,* respectively. The increase in carotenoid content was evidenced by the colour change in the ovary of *P. pelagicus* from light yellow to dark orange. Such deepening of colour with the increase in carotenoid content of the ovary has been reported by Harrison (1990) in decapods and by Diwan & Mohamed (2007) in *F. indicus.* The haemolymph carotenoid content showed an increase till late vitellogenic stage and decreased thereafter (Table 1). Abraham (2005) and Diwan & Mohamed (2007) reported similar observations in *M. monoceros* and *F. indicus,* respectively at the same maturity stages. Harrison (1990) attributed it to the mobilization of carotenoids especially between the hepatopancreas and ovary during vitellogenesis through haemolymph.

The analysis of biochemical constituents during different maturity stages of *P. pelagicus* showed changes in the accumulation of organic reserves in the ovary, hepatopancreas and haemolymph. Considerable reduction was noticed in the moisture content, which could be due to the continuous deposition of organic matter in hepatic and ovarian tissues during maturation. The increase of the biochemical constituents in the haemolymph with the progress of vitellogenesis, indicates its role as a carrier of nutrients between different tissues. The mobilization of protein from the hepatopancreas to the ovary during maturation was demonstrated by a steady decrease of the same in the hepatopancreas. There was a gradual increase of protein in the ovary which reached the maximum at the mature stage.

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