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Biochemical changes in different tissues during yolk synthesis in marine prawn *Penaeus indicus* H. Milne Edwards

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Biochemical changes taking place in ovary, hepatopancreas, muscle and haemolymph of *P. indicus* during ovarian maturation have been studied and quantified. The trends in the fluctuation of metabolites indicate that in ovary there is considerable accumulation of protein, lipid, cholesterol and carotenoids but loss of moisture and nucleic acids. Translocation of these metabolites takes place from the chief synthetic and storage organ, hepatopancreas, via haemolymph. During the initial stages of yolk synthesis, ovary is autosynthetic as indicated by high RNA values and RNA/protein ratio. Subsequently the mode of yolk formation shifts to heterosynthesis mainly through the uptake of lipid from hepatopancreas. RNA/DNA ratio has been found to be useful as an index of ovarian growth.

Oogenesis in crustaceans includes synthesis of nutritive yolk in the ooplasm to meet the basic requirements of embryonic development, independent of the maternal organism[1]. Studies on yolk production in crustaceans have focussed on the potential tissue sources of the yolk components like hepatopancreas, muscle and epidermal adipose tissues[2-7]. In most crustaceans, yolk materials produced in the extra-ovarian sites are transported to the ovary through the haemolymph (heterosynthesis). A recent ultrastructural and histochemical study of the ovary in *Penaeus indicus* has indicated that the biosynthesis of yolk in this penaeid prawn is probably by auto-heterosynthetic means[8]. However the qualitative and quantitative information on the mobilization and build up of organic reserves in relation to reproduction in culturable species of penaeid prawns is lacking. In view of the significance of such information in the scientific management of broodstock in prawn hatcheries, the present investigation on female *P. indicus* has been carried out to quantify variation in protein, carbohydrate, lipid, cholesterol, carotenoid, DNA, RNA and moisture in various tissues like ovary, hepatopancreas, muscle and haemolymph during different maturity stages.

Materials and Methods

Live females of *P. indicus* (120-170 mm total length) were collected from the sea off Cochin by short duration otter trawling. The prawns were then transported live to the laboratory and kept in plastic pools containing seawater (sal. 28-32 $\times 10^{-3}$) and were later segregated according to a 5 point maturity scale[9].

Samples of haemolymph (HL), ovary (OV), hepatopancreas (HP) and muscle (MS) from prawns of all maturity stages were analysed for total protein[10], lipid[11], carbohydrate[12], cholesterol[13] and carotenoid[14] contents. HL samples were collected by direct cardiac puncture using a hypodermic syringe rinsed in an anticoagulent (10% trisodium citrate). OV, HP and MS tissues were analysed for moisture, RNA[15] and DNA[16] contents in addition to the above. Carotenoids were not estimated in MS tissues because of undetectable levels. Dried (70°C) and powdered tissue samples were used for the estimation of protein, lipid, carbohydrate and cholesterol, while for the estimation of moisture, carotenoids, DNA and RNA fresh tissues were used. Six replicates were carried out for each estimation.

Analysis of variance (ANOVA) was performed to test significant differences in biochemical values of a particular metabolite during different maturity stages.

Results and Discussion

The results show cyclic variation and accumulation of organic reserves in HL, OV and HP of *P. indicus* during yolk synthesis.

Proteins—Significant ($P < 0.01$) hike in the protein levels observed (Fig. 1A) during active phases of vitellogenesis in HL as well as OV indicates probable plasmal transport of proteins from an external

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source to the ovary. In the American lobster *Homarus americanus*, total serum protein was in peak levels during the mature stage due to the mobilization of proteins to the ovary[18]. Although the protein content in HP was poor, there was a progressive reduction in protein levels in this organ during vitellogenesis indicating that considerable mobilization of proteins takes place from HP. Other studies[4,19] on yolk protein synthesis in penaeids have shown that ovary is the prime source of yolk proteins. However, in a recent study on *P. vannamei*, Quackenbush[6] reported that HP produces a subunit of egg yolk protein which is transported to OV through HL. He also suggested that egg yolk protein could serve as a lipid shuttle to the developing ovary. Since the quantum of proteins transferred from HP to OV in *P. indicus* is small and as almost all of the lipid yolk is derived from HP, these proteins may also function as lipid carriers. Moreover, an earlier study[8] has shown that pinocytotic vesicles along the oolemmal wall of *P. indicus* aids in active intake of organic material from outside source into oocytes.

Although MS protein did show significant ($P < 0.01$) variation, MS RNA/protein ratio (see Fig. 5A) was uniform throughout indicating that protein synthesis did not take place in MS in relation to yolk synthesis. Earlier studies[17], have also shown that maturity stages did not alter MS biochemical composition of Indian penaeid prawns.

Carbohydrates—Differences in carbohydrate content (Fig. 1B) in HL, OV and HP were significant at 1% level, while in MS, it did not show any significance. In HL, carbohydrate content increased four-fold during stage IV but decreased drastically in spent prawns. Similarly in *Parapenaeopsis hardwickii*[20], it was reported that an incessant hike in HL glucose levels occurs alongwith ovarian development. The trend of HL and hepatic carbohydrate content observed presently indicates translocation of these substances to ovary for utilization in the synthesis of yolk and perhaps also as a fuel during

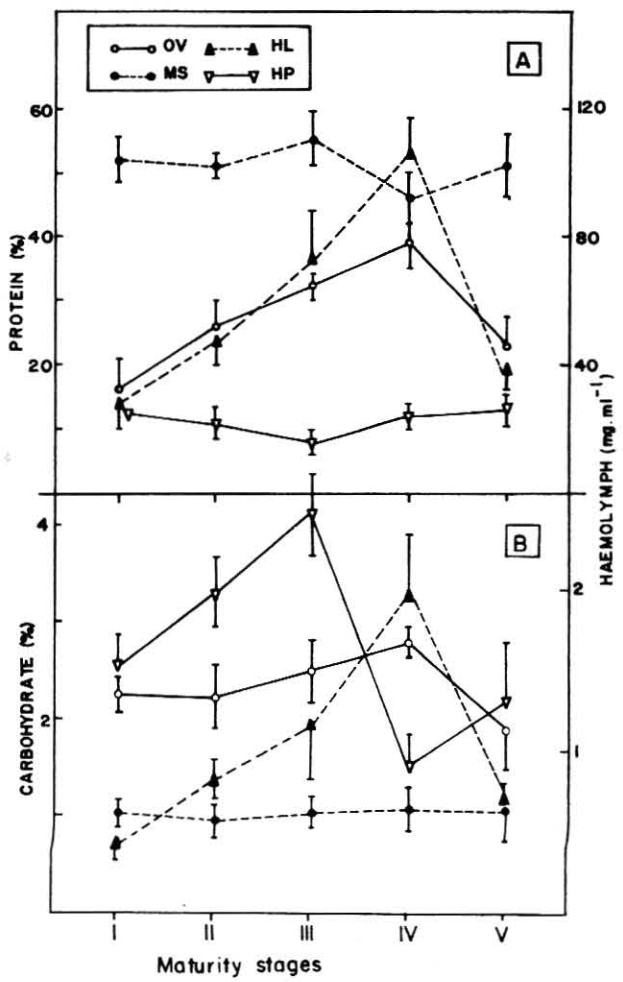

Fig. 1—Trends in variation of protein (A) and carbohydrate (B) in HL, OV, MS and HP during different maturity stages [Vertical lines indicate standard deviation (SD)]

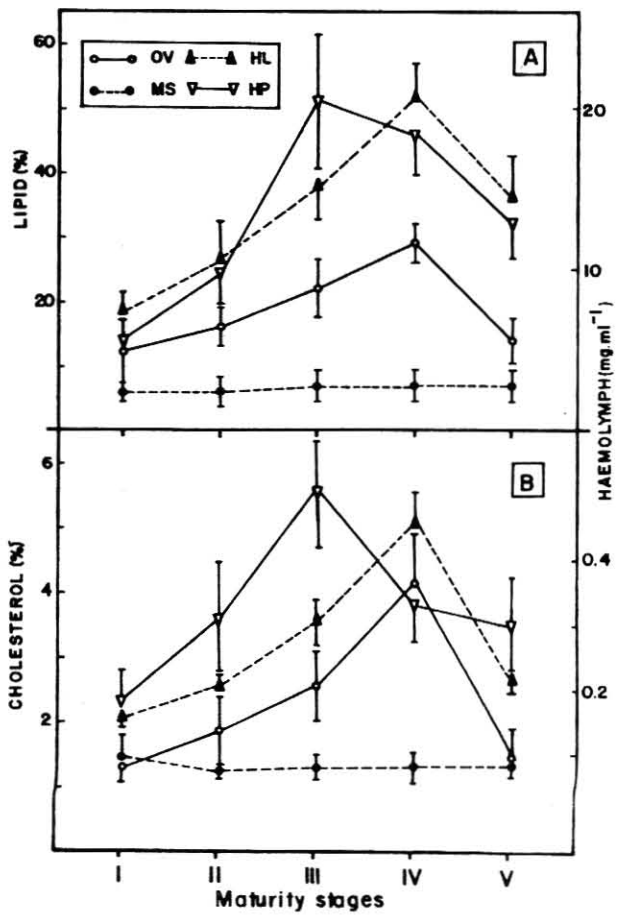

Fig. 2—Trends in variation of lipid (A) and cholesterol (B) in HL, OV, MS and HP during different maturity stages [Vertical lines indicate SD]

the vitellogenic process. There is evidence to show that in *P. notalis* glycogen is mobilized from HP to the gonads during ovarian development[21].

Lipids—Variation in lipid levels (Fig. 2A) in HL, OV and HP were significant ($P<0.01$) indicating mobilization of lipids from HP to OV via HL. In stage V, spent recovery, HL lipid content remained at comparatively high levels due to possible resorption of relict oocytes, which involves retransport of lipid material from the ovary back to the storage site. This may also be due to the rapid rematuration capability of this species[22]. Relatively high lipid levels present in HP of spent prawns also support this view.

Hepatopancreas has been identified as the principal storage site for lipids in crustaceans[23], and in penaeid prawns, origin of ovarian lipids is reported to be the hepatopancreas[24]. Apparently large amount of this stored lipid is mobilized to OV from HP in *P. indicus* during stages II, III and IV through HL. The process of synthesis, accumulation and mobilization of lipid from HP is seemingly continuous as indicated by the peak value (52%) in stage III and the marginal decrease thereafter in stages IV and V. Further, resorption of relict oocytes and rematuration can also account for high lipid content in HP of spent prawns. MS tissues were poor in lipids and no definite trend could be observed.

Cholesterol—HL and OV showed (Fig. 2B) considerable accumulation of cholesterol during vitellogenesis. Concurrently there was a decrease in cholesterol levels after stage III in HP indicating its continuous accumulation and transport to OV via HL. Muscle tissues did not show any significant variations while variations in HL, OV and HP were significant ($P<0.01$). Cholesterol has been demonstrated to be the dominant sterol in the ovary of penaeids[25]. Crustaceans lack the ability to synthesize cholesterol although they are necessary to suppress the permeability of phospholipid membranes[26], and therefore it is presumed that cholesterol present in HP is derived solely from the diet.

Moisture—Moisture (Fig. 3A) was the principal component of ripe ovary although it showed a declining trend in both OV and HP and maximum values were seen in spent prawns. Percentage variations in OV and HP were significant ($P<0.01$) while in MS it was less and not significant ($P>0.01$). In *Metapenaeus affinis* and *Portunus pelagicus*, an inverse relationship was observed between water content and gonad development[30]. It is possible that continued deposition of organic materials in ovarian and hepatic tissues results in loss of water content.

Carotenoids—The deepening colouration of OV during maturation was due to the increase of carotenoid pigments from stage I to IV. The increasing trend of carotenoid content (Fig. 3B) in HL is apparently owing to its transfer from HP to OV. HP was the richest source of carotenoids and peak value was observed in stage III after which the values stabilized between 45 and 47 $\mu g.mg^{-1}$. The hepatopancreas in crustaceans plays a major role in the absorption of carotenoids from food and has been observed to show fluctuation during vitellogenesis[27]. Ceccaldi and Martin[28] also showed that in *Carcinus maenas* carotenoid pigments are transferred from HP into HL during vitellogenesis. However, the precise biological function of carotenoids in crustaceans is still vague[29]. Muscle tissues did not have detectable quantities of carotenoids and differences in HL, OV and HP were significant at 1% level.

Ribonucleic acid—RNA content (Fig. 4A) in OV decreased significantly ($P<0.01$) from an initial peak of 51 $\mu g.mg^{-1}$ in stage I to 16 $\mu g.mg^{-1}$ in stage IV and was restored to the initial concentration in

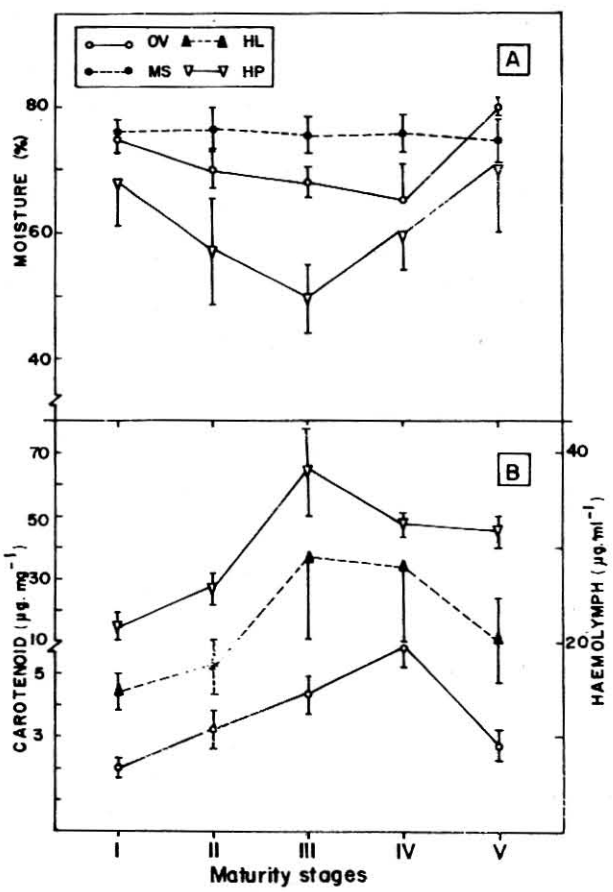

Fig. 3—Trends in variation of moisture (A) and carotenoids (B) in HL, OV, MS and HP during different maturity stages [Vertical lines indicate SD]

stage V. RNA content of MS also showed significant variation ($P<0.05$). In HP it showed significant ($P<0.01$) increase from stage I to IV and sharply decreased in spent animals. RNA/protein ratio in OV decreased to very low levels in the ripe stage (Fig. 5A). High values were observed in stage I and V coinciding with peak protein synthesis. In HP, this ratio peaked in stage III due to high metabolic rates taking place in the organ. In MS, the ratio did not vary with maturation.

Despite their importance to crustacean biology, nucleic acids have been investigated only to a limited extent[31], particularly in relation to reproduction. During the early vitellogenic phase of oocytes, RNA level and RNA/ protein ratio in the ovary of *P. indicus* was consistently high due to the autosynthetic manner of protein synthesis and the same was reduced when heterosynthesis was predominant. The RNA content in tissues is known to be high corresponding to the period of maximum protein synthesis[32]. In ocypodid crabs, oocyte resorption resulted in reduction of RNA levels in the ovary and oogonial proliferation coincided with elevated RNA, DNA and protein contents[33].

Deoxyribonucleic acid—Mean DNA content (Fig. 4B) in OV showed a declining trend which was significant ($P<0.01$) with ovarian maturation and lowest value was noticed in stage IV and highest in stage V. This is probably due to rapid increase in cytoplasmic volume rather than an actual decrease in content. However, in spent prawns, ovaries undergo quick recovery with mitotic multiplication of oogonial cells and this explains the remarkable increase in DNA content during this stage. In MS, the variation was marginal ($P<0.05$) and in HP, there was a gradual build up from stage I to V ($P<0.01$). Hepatic DNA levels were consistently high due to the synthetic activity taking place in them.

In OV, RNA/DNA ratio showed a steady increase, peaking in stage IV and declining in stage V (Fig. 5B). In HP, the ratio peaked in stage III and decreased in later stages. RNA/DNA ratio of MS did not show much fluctuation. RNA/DNA ratios have been frequently used as growth index in

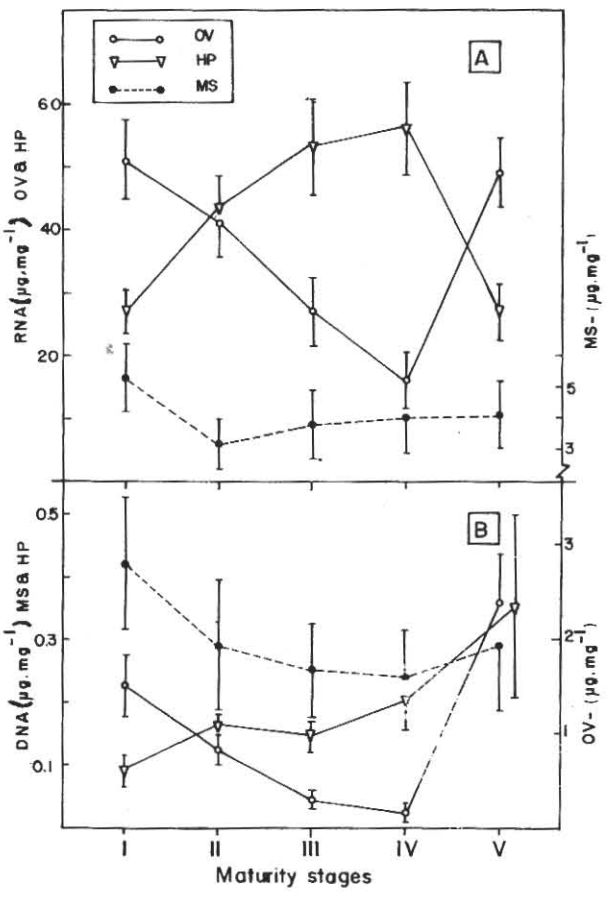

Fig. 4—Trends in variation of RNA (A) and DNA (B) in OV, MS and HP [Vertical lines indicate SD]

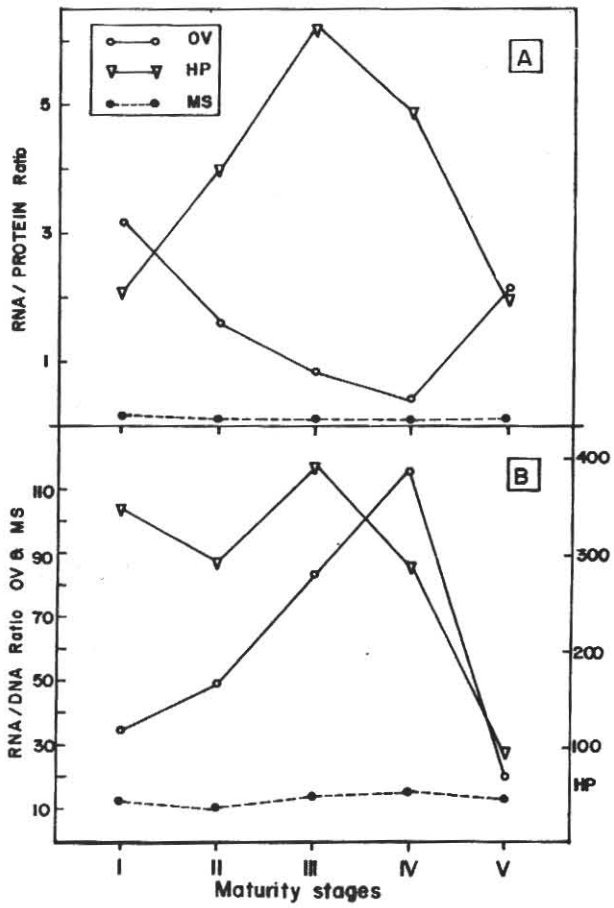

Fig. 5—Variation in RNA/protein ratio (A) and RNA/DNA ratio (B) in OV, MS and HP during different maturity stages.

Table 1—Percentage biochemical composition of mature yolk in *P. indicus*

Metabolite	Percentage	
Moisture	65.41	wet wt
Protein	39.30	dry wt
Lipid	29.14	dry wt
Carbohydrate	2.80	dry wt
Cholesterol	4.14	dry wt
Carotenoid	0.59	wet wt
DNA	0.01	wet wt
RNA	1.63	wet wt

fishes[32] and prawns[34] and the hike in the ratio in the OV indicates its rapid growth by accumulation of yolk.

In crustaceans, composition of yolk varies from species to species[1]. The percentage biochemical composition of mature yolk is given in Table 1. Water formed the principal component of yolk and proteins and lipids constituted the major organic reserves. Other metabolites were present in small quantities only. This study also indicated that a significant portion of proteins and carbohydrates and almost all of lipids, cholesterol and carotenoids found in the yolk were derived from HP via HL. Yolk (protein) synthesis takes place in the ovary only during the early stages of vitellogenesis. Therefore, biosynthesis of yolk is seemingly carried out through dual processes, auto-synthesis and heterosynthesis, and the latter appears to be the predominant one.

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