Indian J. Fish., **51**(1) : 1-9, Jan.-Mar., 2004

Haemolymph proteins of the Indian white shrimp, *Penaeus indicus* (H. Milne Edwards) II. Moult stages and ovarian development

P. LAXMILATHA AND A. LAXMINARAYANA

Central Marine Fisheries Research Institute, Cochin 682 018, Kerala, India

ABSTRACT

Haemolymph proteins of the Indian white shrimp, *Penaeus indicus* in various stages of the moult cycle and ovarian development were characterized by Sodium dodecyl sulphate polyacrylamide gel discontinuous electrophoresis (SDS-PAGE). No specific protein or polypeptide was detected during the various moult stages. In the females with developing and mature ovary, a distinct haemolymph protein pattern was observed. The presence of female specific proteins in the haemolymph is described. The FSPs stained positive for PAS, Oil Red O and Alizarin Red. The peculiar pattern of hemocyanin fractions during vitellogenesis is discussed.

Introduction

Vitellogenin (VG), is known to be a blood serum precursor of the egg yolk protein, secreted into the haemolymph during the process of vitellogenesis. This circulating ovarian precursor is also termed as female specific protein (FSP). When vitellogenin enters the oocytes, it is known as vitellin or lipovitellin (LV). In crustacea, vitellogenin in the haemolymph is reported to vary during the reproductive cycle (Ceccaldi and Martin, 1969; Horn and Kerr, 1969; Ceccaldi, 1971; Fielder *et al.*, 1971; Picaud, 1971, 1978b; Wolin *et al.*, 1973; Fyffe and Connor, 1974; Saiag *et al.*, 1979; Derelle *et al.*, 1986; Munuswamy and Subramoniam, 1987; Tom *et al.*,1987a, b, Yano and Chinzei, 1987: Nelson *et al.*,1988; Browdy *et al.*, 1990, Fainzilber *et al.*, 1992; Komatsu and Ando, 1992; Shafir *et al.*, 1992).

In the present study, the haemolymph protein pattern of *Penaeus indicus* H. Milne Edwards (Crustacea: Decapoda: Penaeidae), in relation to the various moult stages and ovarian development was characterized by SDS-PAGE.

Materials and methods

Specimens used for the present study were collected from the brackishwater aquaculture ponds at Narakkal as well as from offshore waters and transported to the laboratory at Central Marine Fisheries Research Institute, Cochin and maintained for acclimatization in 23-25% sea water in 1 ton fibre glass tanks provided with continuous aeration. The haemolymph was extracted by piercing a 22-guage needle attached to 1 ml syringe, directly into the heart, just below the cephalothorax. Tri-sodium citrate (3%) was used as anticoagulant. The samples were stored at 4° C until further use.

Haemolymph proteins of *P. indicus* in size range 100-120 mm, in various stages of moult: (early postmoult, late postmoult, intermoult, mid premoult, late premoult, premoult (A, B, C, D_1', D_1'', D_1''', D_{2-3})) and vitellogenesis in immature, early maturing, late maturing, fully mature and spent (Stage I, II, III, IV and V) specimens were characterized by SDS-PAGE (Laxmilatha and Laxminarayana, 2003).

Results

Haemolymph protein variations with respect to moult cycle

The haemolymph protein pattern was identical in the different moult stages. The observed minor differences were due to intraindividual variations rather than related to any particular moult stage. No significant variation in the protein pattern could be detected. The quantitative increase in the total protein content in the haemolymph from the post moult stages (A and B) through intermoult (C) to late premoult (D_1'') and thereafter the sudden decline just prior to ecdysis (D_{2-3}) did not manifest in the haemolymph protein pattern.

Haemolymph protein pattern during ovarian development

A comparision of haemolymph protein fractions separated from individuals in different stages of ovarian maturity *vis-a vis* male haemolymph protein pattern is represented in Fig.1. Altogether 13 fractions were separated, wherein the densely stained fast hemocyanin fraction represented high protein content. In the females, the number of fractions varied from 12-15 from stage I to stage V (spent). In stage I, 14 fractions were separated which include intensely stained fractions 4, 5, 6, 7, 8 and 9 . This was obvious in stage II also. Fractions 4, 6, 7, 8 and 9 were

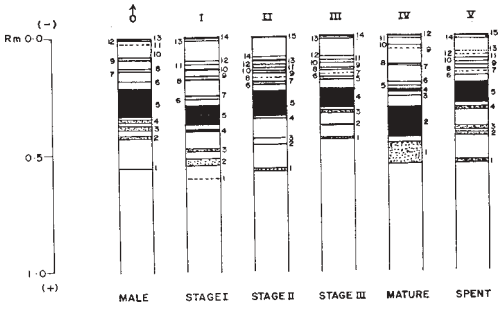

Fig. 1. Comparative electropherograms of haemolymph proteins of *Penaeus indicus* in different stages of ovarian development, along with male haemolymph proteins.

highly specific in these stages. . Since these are prominent only during vitellogenesis they owe their significant functional importance as Yolk proteins or Female specific proteins (FSP). These fractions however were less obvious in stage III and were highly disoriented in the mature and spent stage.

The specific nature of the Female specific proteins (vitellogenins) is indicated in Fig. 2 and 3. These fractions were glycoproteic in nature, stained positively with PAS and became intense during the development of ovary. In stage I, fractions 4, 5, 6 ,7, 8, 9, 10, 13 and 14 were glycoproteins out of which the fractions 4, 5, 7,and 8 were also lipoproteins. The copper binding fractions revealed a peculiar pattern , in that only one fraction stained positively with Rubeanic acid , reflecting an altered role and structure of the hemocyanin molecule during vitellogenesis.

In stage II, the glycoproteins were predominant. The fractions 4, 5, 6, 7, 9, 11, 12 and 15 stained for PAS, while lipoproteins were similar to those in stage I; i.e., fractions 4, 5, 6 and 7 were positive for Oil Red O. The calcium binding proteins were the same as the glycoproteins. This indicate that the

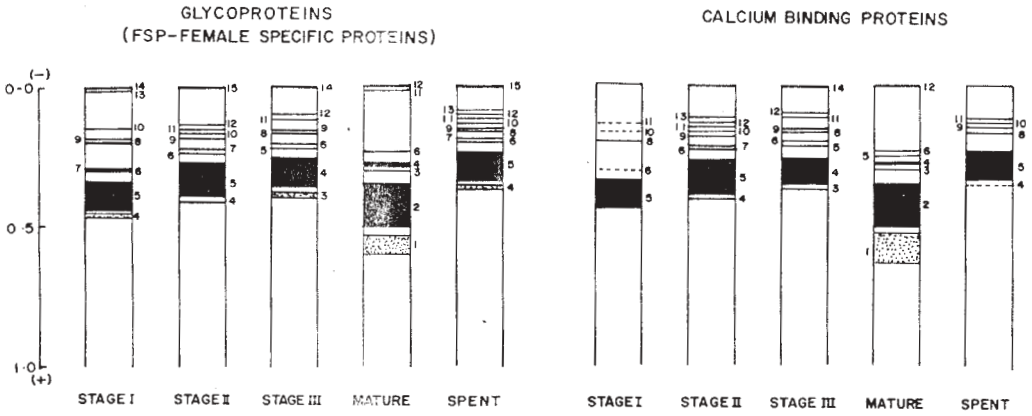

Fig. 2. Comparative electropherograms of haemolymph glycoproteins and calcium binding proteins of *Penaeus indicus*, in different stages of ovarian development.

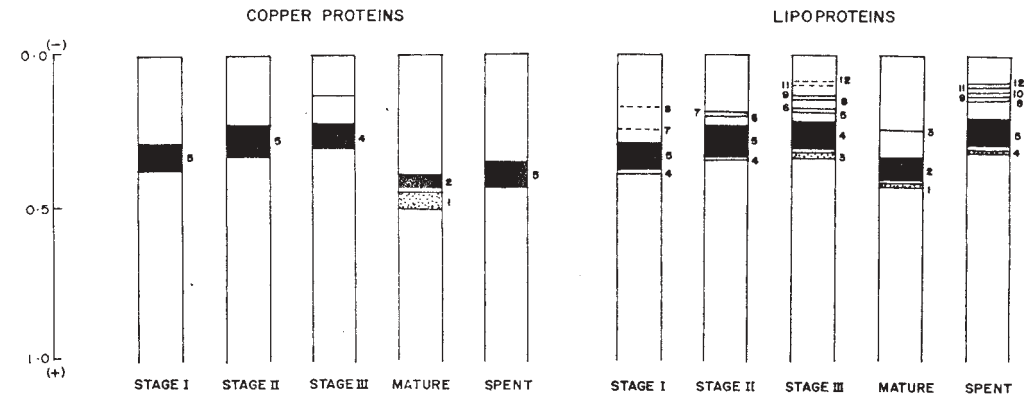

Fig. 3. Comparative electropherograms of haemolymph copper binding proteins and lipoproteins of *Penaeus indicus*, in different stages of ovarian development.

FSPs are complex proteins with calcium modulating the process of vitellogenesis. In Stage II also the copper- bearing fraction was represented by a single (fast moving) fraction.

In stage III, the intensity and number of glycoproteins increased. They represented fractions 3, 4, 5, 6, 8, 9, 11, 12 and 14. The lipoproteins also increased in this stage represented by fractions 3, 4, 5, 6, 8, 9, 11,and 12. The calcium binding proteins were similar to the glycoproteins 3, 4, 5, 6, 8, 9, 11,12 and 14 stressing the role of calcium in vitellogenesis. The slow moving hemocyanin fraction which was absent in the earlier stages reoccurred in this stage.

In fully mature stage, there was a drastic change in the haemolymph protein pattern. Along with a reduction in the female specific proteins there was an increase in protein content compared to other stages as represented by the broad and intensely stained fast hemocyanin fraction. In this stage, again a peculiar reorientation of the copper bearing fractions occurred. The slow moving fraction was replaced by a fast moving, broader fraction just below the

normally occurring fast hemocyanin fraction. The glycoproteins and calcium binding fractions were 1, 2, 3, 4, 6 and 12 while lipoproteins were 1, 2 and 3.

In the spent stage (V), again there was an altered haemolymph protein pattern. The female specific proteins were present but reduced in intensity compared to stages I, II and III. The glycoproteins were 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 15. The calcium fractions had reduced and were represented by 4, 5, 8, 9, 10 and 11. The lipoproteins comprised fractions 4, 5, 8, 9, 10, 11 and 12. The only copper-binding fraction noticed in this stage was fraction 5 .

Discussion

In *P. indicus*, haemolymph protein characterization by SDS-PAGE did not reflect any significant qualitative variation in the protein pattern during moult cycle. No specific or characteristic moult related fraction could be traced throughout the moult cycle. Applying starch gel electrophoresis and immunodiffusion techniques Barlow and Ridgway (1969) observed a relative shift in the concentration of certain antigens in lobster, *Homarus americanus* sera with changes in the moult cycle. In this species, both sexes possessed an antigen that disappeared about one month after moult and appeared again in about four weeks. Using cellulose acetate electrophoresis, Busselin (1970) traced protein pattern of haemolymph of *Cancer maenas* during moult cycle. He observed that moulting causes a four to five fold decrease in the concentration of hemocyanin. But the premoult levels were restored progressively during the intermoult. At ecdysis, glycoprotein fraction I, disappeared completely and reappeared after 10 days. The concentration of this fraction was found to be highly dependent on the nutritional status of that crab species. In *Maia squinado,* the hemocyanin fraction disappeared during premoult and post moult stages and reappeared during intermoult (Zuckerkandl, 1956). In *C. mediterraneous*, immunochemical analysis revealed two groups of fractions at the end of intermoult which increased at the beginning of D_0 only, which disappeared by the time two new hypodermal fractions appeared (Herberts *et al.*, 1978). By two dimensional electrophoresis of *Astacus leptodactylus* haemolymph Vranckx and Durliat (1976) recorded variations during moult cycle. Of the 8 fractions observed, which included hemocyanin and fibrigonen in serum and plasma, fraction II showed a characteristic pattern. It was stable during C_4 and increased steadily from D_0 to D_1 and again decreased upto D_3 and postmoult A. This suggested that in lobsters, crayfishes and crabs, the moult cycle is an extended one (almost one year) and leads to synthesis of specific moult related proteins. Also the appearance and disappearance of particular polypeptides could be attributed to the obligatory starvation prior to ecdysis (Barlow and Ridgway, 1969; Busselin, 1970; Herberts *et al.*,1978; Vranckx and Durliat, 1976). On the other hand, in shrimps, particularly penaeids, the moult cycle is comparatively shorter (15-20 days) and quantitative variations observed are dilution of blood proteins by water uptake prior to ecdysis, rather than an increase or decrease in protein content in the haemolymph. (Dall, 1964 a,b, 1974; Djangmah 1970; Djangmah and Grove 1970). It is also probable that more refined electrophoretic techniques such as two dimensional immunoelectrophoresis or ultrathin IEF may aid in detection of specific polypeptides, in this species, if any,

involved in the moulting process, which might have escaped detection in SDS-PAGE.

In *P. indicus*, the presence of Female specific proteins (FSP) in the haemolymph could be detected during the different stages of ovarian maturity. The female haemolymph revealed the presence of FSP in the early developing stages (I & II) and increased in stage III. The FSP increased in intensity and disappeared partially during the fully mature stage and reappeared in the spent stage although with reduced intensity. These fractions (4, 7, 8, 9) stain for PAS and Oil Red O confirming their glyco-protein nature. The pattern strongly reflects the mode of utilization of lipovitellin which is believed to be synthesised by the ovary and rarely by extraovarian tissues or through the haemolymph in the form of vitellogenin, transferred to the developing oocytes. Thus, in the fully mature stage, the vitellogenin is absorbed by the developing oocytes as lipovitellin, thereby resulting in the altered pattern of haemolymph FSP in the fully mature stage. The reappearance of FSP in the spent stage (although decreased intensity) indicates probably residual or unabsorbed vitellogenin. Studies on other crustaceans, including decapods, have revealed one or more ovarian lipovitellins with immunologically corresponding haemolymph vitellogenins (Wolin *et al.,* 1973; Fyffe and Connor, 1974; Durliat, 1984; Nelson *et al.,* 1988). The electrophoretic pattern of haemolymph and ovarian extract of *Pandalus kessleri* on SDS-PAGE revealed two strong bands in both cases. The molecular weight of the strong bands was estimated to be 68-80 KD for female haemolymph and 78-110 KD for ovarian extract (Quinitio *et al.,*1989). In *Porcellio dilatatus*, completely identical reaction was demonstrated by immunodiffusion between vitellin and vitellogenin (Picaud, 1978a,b). The presence of FSP in the haemolymph of females during vitellogenenis was demonstrated by PAGE in *Ligia oceanica* and *Orchestia gammarella* (Picaud 1971; Junera *et al.,* 1974). In *Macrobrachium rosenbergii*, purified vitellin gave a positive reaction by ELISA titration with vitellogenin also (*Derelle etal.,*1986). High density lipoproteins (HDL) was revealed by differential density gradient centrifugation in *Macrobrachium rosenbergii,* while crayfish *Ibacus ciliatus* revealed both HDL and low density lipoproteins (Komatsu and Ando, 1992). Munuswamy and Subramoniam (1987) by PAGE revealed the appearance of FSP (glycoproteins) in stage III of maturity and mature stage in the fairy shrimp *Streptocephalus dichotomous.* In *Squilla mantis* , SDS-PAGE revealed three female specific protein fractions in the haemolymph and was considered vitellogenins since their amount increased with ovarian maturity. In *Homarus vulgaris,* cellulose acetate electrophoresis and immunodiffusion revealed a female specific component in the haemolymph during ovarian development (Barlow and Ridgway, 1969).

Female specific proteins (FSP) associated with ovarian development have been detected in the haemolymph of crabs too. Horn and Kerr (1969) detected a lipoprotein in female sera of *Callinectes sapidus.* In *Uca pugilator*, Fielder *et al.*(1971) found the FSP in haemolymph with developing oocytes and the lipoprotein was detected in both, the light and dark patch variants. Maguire and Fielder (1975) identified a glycoprotein which increased in the haemolymph as ovarian development progressed in portunid crab, *Portunus*

pelagicus. It occurred in a few males as well and hence cannot be considered as FSP. In the hermit crab, *Clibanarius clibanarius*, the FSP in the hemolymph, has 3 fractions (3,4,5) which separate discretely in ovarian stages I and II. The fraction 5 is the only PAS staining fraction in stage II and disappearing in stage III when its full intensity is displayed in the ovary. During resorption, the pattern is the same as in stage III and resembles the male hemolymph pattern. This is observed in *P. indicus* also where in the FSP (fractions 4,7,8 and 9) partially dispappears in the fully mature stage and reappears in the spent stage probably for the next cycle. In *Parapenaeopsis longirostris*, Tom *et al.*, (1987a, b) identified one protein band in the purified vitellin. Vitellin and its possible precursors were localised by means of immunochemical and immunohistochemical methods. The significant role of haemolymnph in transporting Vg between its processing sites was confirmed in *Penaeus semisulcatus* by labelled methionine (Browdy *et al.*, 1990; Fainzilber *et al.*, 1992; Shafir *et al.*, 1992). Further immunological studies on FSP in *P.indicus* may reveal its immunological identity with lipovitellin in the ovary.

In *Penaeus indicus*, the copper-bearing proteins-hemocyanins behave differently during the ovarian developing stages. In this case, the two copper-binding components, present in all individuals examined, irrespective of sex, size and moult stages, exhibit a peculiar pattern during the vitellogenic process. Only one fast moving component is present in stage I, II and III of ovarian maturity. In fully mature individual, the pattern was slightly different, with two broad, diffuse components - one with same mobility as seen in normal cases, and the other with greater mobility than the first component. In the spent stage, the two components as seen in normal case was observed. This phenomenon was not recorded in any other penaeid species. Wache *et al.* (1988) studied the structural changes of hemocyanin during early development in the crab, *Cancer productus* and found two hexamer hemocyanins in the oocytes having an exactly same structure as that of molecules in adult hemocyanin. She suggested that the oocyte probably takes up the maternal hemocyanin through endocytosis. Probably this is the case in *P. indicus* too and it will be of interest to know which fraction is involved in this function. Perhaps the hemocyanin functions to facilitate oxygen diffusion to the metabolising oocytes. Similar occurrence of hemocyanin in early development stages has been reported in *Astacus leptodactylus* (Durliat, 1984) and *C. maenas* (Busselin, 1971). The presence of hemocyanin in the oocytes of *P. indicus* has not been elucidated in the present study, and if present, it will be of interest to compare the functional properties of the embryo hemocyanin with those of adult hemocyanins.

The female specific protein (Vitellogenin) is a complex protein staining positive for calcium. The increase in calcium binding proteins during vitellogensis stresses the role of calcium in transporting vitellogenin to the developing oocytes through the haemolymph. There is no record of qualitative estimation of calcium binding proteins during vitellogenesis, in the haemolymph, in other species, although the role of calcium in transporting vitellogenin to developing oocytes is well recorded in fishes (Bjornsson and Hanx, 1985; Tinsley, 1985). In *P. indicus* almost all the haemolymph proteins are of high molecular weight including the

lipoprotein fractions, as apparent by their low relative mobility.

Acknowledgements

This study formed part of the Ph. D research work of the first author for which Senior Research Fellowship was awarded by the Indian Council of Agricultural Research, New Delhi. The facilities provided by the Central Marine Fisheries Research Institute, Cochin is gratefully acknowledged.

References

Barlow, J and G.J. Ridgway 1969. Changes in serum protein during the molt and reproductive cycles of the American lobster (*Homarus americanus*). *J. Fish. Res. Bd.* Canada, **6** (8): 2101-2109.

Bjornsson, B. Th and C. Hanx 1985. Distribution of calcium, magnesium and inorganic phosphate in plasma of estradiol-17 treated trout. *J. Comp. Physiol*., **15** B: 347-352.

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

Busselin, P. 1970. Effects of moulting cycle and nutritional conditions on the haemolymph proteins in *Carcinus maenas*. *Comp. Biochem. Physiol*., **40A**: 155-162.

Busselin, P. 1971. The electrophoretic heterogeneity of *Carcinus maenas* hemocyanin. *Arch. Biochem. Biophys.*, **137**: 415-420.

Ceccaldi, P. 1971. Proteines de l'hemolymphe de *Scyllarus arctus* (Crustace Decapode). *C.R. Soc. Biol*., **2**: 364

Ceccaldi, H.J and J.L. M Martin 1969. Evolution des proteins de hemolymphe chez carcinus maenasi durant l'ovogenese. *C.R. Soc. Biol*., **163:** 2638-2641.

Dall, W. 1964a. Studies on the physiology of a shrimp, *Metapenaeus mastersii* (Haswell) (Crustacea: Decapoda: Penaeidae). *Australian J. Mar. Freshwater Res*., **15**: 145-161.

Dall, W. 1964b. Studies on the physiology of a shrimp, *Metapenaeus* sp. (Crustacea: Decapoda: Penaeidae). IV. Carbohydrate metabolism. *Australian J. Mar. Freshw. Res*., **16**: 163-18.

Dall, W. 1974. Indices of nutritional state in the Western rock lobster *Panulirus longipes* (Milne Edwards) I. Blood and tissue constituents and water content. *J. Exp. Mar. Biol. Ecol.*, **16**: 167-180.

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

Djanmah, J. S. 1970. The effects of feeding and starvation on copper in the blood and hepatopancreas and on blood proteins of *Crangon vulgaris* (Fabricius). *Comp. Biochem Physiol*., **32**: 709-731.

Djangmah, J. S and D. J. Grove 1970. Blood and hepatopancreas copper in *Crangon vulgaris* (Fabricius). *Comp. Biochem. Physiol*., **32**: 733-745.

Durliat, M. 1985. Clotting processes in Crutacea (Decapode). *Biol. Rev*., **60**: 473-498.

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

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

Feilder, D. R., K. Ranga Rao and M. Fingerman 1971. A female limited lipoprotein and the diversity of hemocyanin components in the dimorphic variants of the fiddler crab,

Uca pugilator as revealed by disc electrophoresis. *Comp. Biochem. Physiol.*, **39B**: 291-297.

Herberts, C., Nicole Andrieux et Jacques de Freschville 1978. Variations des proteins de l'hemolymphe et de l'hypoderme an cours du cycle de mue chez *Carcinus mediterraneous* czerniavsky analyses electrophoretique et immunochimique. *Canadian J. Zool.*, **56** (8): 1735-1743.

Horn, E. C. and M. S. Kerr 1969. The haemolymph proteins of the blue crab, *Callinectes sapidus*- I. Hemocyanins and certain other major proteins constituents. *Comp. Biochem Physiol.*, **29** (53): 335-346.

Junera, H., M. M. Jean-Jacques Meusy et Yvon croiselle 1974. Etude comparee de la "fraction proteique femelle" dans l'hemolymph et dans l'ovaire du crustace, Amphipoda *Orchestia gammarella* pallas par electrophorese en gel de polyacrylamide. *C. R. Acad. Sci.* Paris., **278** SerieD: 655- 658.

Komatsu, M. and S. Ando 1992. Isolation of crustacean egg yolk lipoproteins by differential density gradient ultracentrifugation. *Comp. Biochem. Physiol.*, **103**B (2): 363-368.

Laxmilatha, P. and A. Laxminarayrna2003. Haemolymph proteins of the Indian white shrimp, *Penaeus indicus* (H.Milne Edwards). *Indian J.Fish.*, **50** (3): 279-290.

Maguire, S. H. P. and D.R. Feilder 1975. Disc electrophoresis of the hemolymph proteins of some portunid crabs. *Comp. Biochem. Physiol.*, **52A**: 39-42.

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

Nelson, K., B. Heyer, E. Jhonson, D. Hedgecock and E.S. Chang 1988. Vitellogenessis in female lobsters (*Homarus americanus*). *Comp. Biochem. Physiol.*, **60B**: 809-821.

Picaud, J.L. 1971. Mis en evidence de proteins de type femelle et d'une proteins specific du functionment ovarian par electrophorese sur gel de polyacrylamide de l'hemolymphe et de broyats d'ovares de *Ligia oceanica*. *L. C.R. Acad Sci*, Paris, **273** (14): 1214-1216.

Picaud, J.L. 1978a. Contribution a'le' tude des proprietes physio-chimiques des proteins specifiques femelles de *Porcellio dilatatus* Brandt (crustace Isopode, Oniscoide) . *C.R. Soc. Biol.*, ***172*** (2): 299-303.

Picaud, J.L. 1978b. Proteins antigeniques des proteins specifiques femelles chez quelques crustaces isopods. *C.R Soc. Biol.*, **172** (2): 320-324.

Quinitio, E.T., A. Hara., K. Yamauchi, T. Mizushima and F. Fuji 1989. Identification and characterisation of vitellin in a hermaphrodite shrimp, *Pandalus kessleri*. *Comp. Biochem. Physiol.*, **94B**(3) : 445-451.

Saiag, B., J. J. Mustel and Y. Croiselle 1979. Female specific proteins (Vitellogenin and Lipovitellins) in *Orchestia gammarella* (Crustacea, Amphipoda): Isolation and identification by means of glutaraldehyde polymerised antibodies. *Comp. Biochem. Physiol.*, **64B:** 285-288.

Shafir, S., M. Ovadia and M. Tom 1992. *In vivo* incorporation of labelled Methionine into protein vitellogenin and vitellin in females of penaied shrimp *Penaeus semisulcatus* de haan. *Biol. Bull.*, **183**:242-247.

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

Tom, M., M. Goren and M. Ovadia 1987a. Localization of the vitellin and its

possible precursors in various organs of *Parapenaeus longirostris* (Crustacea, Decapoda, Penaeidae). *Int. J. Invert. Repr. Develop*., **12**: 1-12.

Tom, M., M. Goren and M. Ovadia 1987b. Purification and partial characterization of vitellin from the ovaries of *Parapenaeus longirostris* (Crustacea, Decapoda, Penaeidae). *Comp. Biochem. Physiol.*, **87B** (1)**:** 17-23.

Vrankx, R and Michelle Durliat 1976. Analyse immunologoque des variations des proteinsde l' hemolymphe d' *Astacus leptodactylus* pendant la premue. *C.R. Acad. Sci.* , **282** D: 1305-1308.

Wache, S., N. B. Terwilliger and R. C. Terwilliger 1988. Hemocyanin structure changes during early development of the crab, *Cancer productus. J. Exp. Zool.,* **247:** 23-32.

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

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

Zuckerkandl, E. 1956. La variation an cours du cycle d' intermue des fractions proteiques de l' hemolymph de *Maia squinado* separees par electrophores. *C. R. des seances de la Soc. de biol et de se Filiales*, **150**: 39-41.