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Hepatopancreatic contributions of lipids and carotenoids to vitellogenesis in the intertidal anomuran crab, *Emerita asiatica* (Milne Edwards)

Persia Jothy T[1], Rajesh Kannan R[1] & Subramoniam T[2]*

[1]Molecular and Nanomedicine Research Unit, Centre for Nanoscience and Nanotechnology, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India
[2]Department of Zoology, University of Madras, Guindy Campus, Chennai, Tamil Nadu, India

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In decapod crustaceans, lipids and the associated carotenoid pigments form an integral part of yolk to serve as nutrients during embryogenesis. This study reports on the analysis of different lipid classes and the major carotenoids in the ovary, hepatopancreas and hemolymph and their fluctuation during different phases of ovarian maturation in an anomuran crab, *Emerita asiatica.* Neutral lipids including triglycerides (TG) and free fatty acids (FFA) formed the bulk of ovarian lipids. Important fatty acids are Saturated fatty acids (SFA) 16:0 and 18:0, Monounsaturated fatty acids (MUFA) 16:1n7 and 18:1n9, and Polyunsaturated fatty acids (PUFA) 20:5n3 and 22:6n3. While phospholipids increased during maturation, glycolipids decreased. Cholesterol level in ovary increased initially, but declined during later stages. Dominant pigments, β-carotene and astaxanthin, steadily increased during ovarian maturation within the ovary, although canthaxanthin declined drastically towards last stage. In hepatopancreas, however, TG and FFA showed gradual decrease during maturation. Palmitic acid, palmitoleic acid and eicosapentaenoic acid are the predominant fatty acids in hepatopancreas, showing a steady decline during ovarian maturation. Other lipid classes such as glycolipids also showed a decline in hepatopancreas. Both β-carotene and astaxanthin in hepatopancreas declined from the first stage of ovarian development, suggesting translocation to ovary. The overall metabolic changes of lipids and carotenoids in hepatopancreas, hemolymph and ovary are indicative of their accumulation within developing eggs to provide metabolic energy and substrates for membrane formation, and to serve as precursors for pigment formation respectively, during embryogenesis.

Keywords: Astaxanthin, β-carotene, Cholesterol, Fatty acids, Glycolipids, Phospholipid, Steroidogenesis

In recent years, the mechanism and control of vitellogenesis in decapod crustaceans have been studied extensively, thanks to the increased attention on the broodstock production of shrimps and other economically significant decapod crustaceans[1]. Although vitellogenin (Vg) synthesis and its transcriptional control by gonadotropic hormones is well documented for hepatopancreas, its role in the contribution of lipids, which constitutes as much as 30% in the Vg molecule has not received adequate attention. The de novo synthesis of certain essential lipids, including polyunsaturated fatty acids (PUFAs) that are essential for growth and reproduction[2], is limited in crustaceans, and hence are acquired through the diet[3]. Phosphatidylcholine (PC) and triacylglycerol are the other predominant lipids in shrimp ovaries that are required for embryogenesis, and hatching to produce healthy nauplii[4].

Another characteristic feature of decapod yolk protein is the conjugation of carotenoid pigments, imparting a variety of colours to the spawned egg. In decapod eggs, carotenoids are esterified to the fatty acids of the vitellogenin/lipovitellin molecules[5]. However, we have only limited knowledge about their synthesis in hepatopancreas and their final transport to the ovary. Previous studies have indicated that carotenoids are absorbed from dietary pigments such as α and β carotene by hepatopancreas and then transported to the ovary by binding to Vg[6].

The present study endeavours to investigate the quantitative fluctuation found in the lipids and major carotenoids in the ovary during different stages of ovarian maturation in the anomuran crab, *Emerita asiatica*. Since hepatopancreas is known to be the source of lipids and carotenoids in crustaceans, analysis of these compounds in the hepatopancreas corresponding to ovarian developmental stages is expected to throw light on their possible transfer to

*Correspondence:
E-Mail: thanusub@yahoo.com

ovary during vitellogenesis. Furthermore, changes in the hemolymph lipids and carotenoids have been followed during ovarian maturation stages to determine their role in the transportation of these compounds from hepatopancreas to ovary.

Materials and Methods

Experimental animal

Emerita asiatica used in this study were collected from a clean intertidal zone of Panayur Beach, Chennai Coast (12°53'00.1"N 80°15'07.9"E). Minimum of 40 crabs were handpicked per collection and brought to the laboratory. The females were identified by the occurrence of three pairs of pleopods. *E. asiatica* ranging in size 10-33 mm carapace length (CL) were collected. The carapace length was measured from the posterior margin of the carapace along the middorsal line to the tip of the rostrum. Collections were made during the day time.

Classification of ovarian stages

Four different ovarian stages of *E. asiatica* were classified as stage I–IV, based on the morphological characteristics, such as colour, oocyte diameter, and gonadosomatic index (GSI=gonad weight/body weight×100). When the embryogenesis occurs in the pleopod, there is a concurrent development of oocytes in the ovary. This condition is helpful in the ovarian classification by merely observing the molt stages and embryo developmental stages in the brood. Further observations have revealed that in *E. asiatica*, molting cycle is closely related to ovarian cycle in such a way that the early premolt stage (D_1) commences during stage IV of ovary maturation[7]. We have followed these criteria in staging the ovarian development in this study.

Sample preparation

The crabs were washed thoroughly with filtered sea water to remove the adhering sand particles. The hemolymph was collected from females by piercing the arthrodial membrane of the last appendage using a fine syringe. Approximately, 50-300 mg of ovarian and hepatopancreatic tissues from three individual crabs at each ovarian stage were taken for total lipid, fatty acid and carotenoid extraction. All tissues were washed thoroughly with 0.9 N saline solution and stored at –20°C for further analysis.

Lipid and carotenoid analysis

The total lipid, cholesterol, glycolipids, phospholipids and fatty acid methyl esters (FAME) of the hepatopancreas, ovary and haemolymph during four stages of ovarian maturation were determined using standard analytical procedures[8]. Lipid from egg was extracted by the method of Folch *et al*.[9] using chloroform: methanol solvent (2:1 v/v) and the extract was used for the quantitative analysis of different lipids. Total lipid was estimated by sulphophosphovanillin method[33], Cholesterol by Zak *et al*.[34], Phospholipid (PL) by Rousar *et al*.[35], Triglyceride by Van Handel *et al*.[36] and Glycolipid by Roughan & Batt[37]. FAME composition was verified by gas chromatography and carotenoid content by high performance liquid chromatography(HPLC) as done by Persia *et al*.[8].

Fatty acid Methyl Ester analysis

The fatty acid (FA) analysis had been carried out using gas chromatography (Shimadzu GC- 2010 PLUS), equipped with an auto sampler. The fatty acid methyl esters (FAME) of sample were prepared by the saponification of lipid extracts using 10ml of 0.5 M NaOH-CH3OH solution at 75°C for 15 min, which was further subjected to methanolysis process using 4% H_2SO_4 in methanol at 75°C for 45 min. FAME (Fatty acid methyl ester) was analytically quantified with the support of flame ionization detection (FID) by injecting a sample through the fused silica capillary column (100.0 m×136.0.25 mm×0.50 μm). The injector and detector temperature were put at 250°C and initial column temperature at 100°C for 4 min, temperature was increased at a rate of 3°C per minute up to 240°C and kept in hold for 15 min until all the injected FAME had been eluted. The total runtime for sample and standard was 65.67min. Helium is used as the carrier gas with the flow rate at 30 mL per min. The peaks were analysed by comparing the sample retention times and areas with known value of standards (Sigma-Aldrich Co., St. Louis, MO, USA).

HPLC analysis for carotenoids

A Waters chromatograph, equipped with a photo diode array detector, was used for the analysis of the various carotenoids, with data collected by Empower 2. Samples were filtered through a 0.45 μm membrane filter to remove particulate residues and 10 μl was injected onto HPLC with a reversed phase column (250 * 4.6 mm, 5 μm), protected by a guard column. Following a series of experiments with the flow rates of 1ml/min and spectra covering a range from 300-700 nm are measured, the specific conditions was optimized with all standard carotenoids and applied for the sample extracts from ovary and hepatopancreas.

All three compounds were successfully eluted within 20 min runtime.

Statistical analysis

Levels of different lipids, carotenoids and fatty acids in hepatopancreas and ovary during ovarian stages were subjected to statistical analysis by Mean ± SD[10], and presented as mean ± standard deviation. One-way ANOVA was used to evaluate significant differences in different parameters of the experimental study. The level of significance was set at $P<0.05$ and Tukey's multiple comparison test was used to determine which of the stages was significantly different from each other. The SPSS software (version 20.0) was used for analysis.

Results and Discussion

Table 1 summarises data on the lipid content of ovary during different developmental stages in *E.asiatica.* Triglyceride (TG) and cholesterol formed the predominant lipid components of the ovaries in all stages of vitellogenesis. TG increased from 74.72±17.03 to 89.40±10.78 mg/g in the final stage with a statistical significance ($P<0.05$). A similar pattern was observed for phospholipids (PL) (0.94±0.01 to 1.18±0.18 mg/g).As shown in Table 1, the relative proportion of PLs increased from 0.47% in stage I to 0.66% in stage IV ovary. Conversely, hepatopancreatic PL decreased from 0.93% in stage I to 0.43% in stage IV ovary. This reciprocal relationship of PL in ovary and hepatopancreas may suggest their transfer from the latter to the ovary during maturation. Evidently, PLs synthesized within the hepatopancreas would be conjugated to the nascent Vg and then transported through the hemolymph to the ovary. Conversely, cholesterol level in the ovary decreased significantly from 20.49±0.63 mg/g in stage I to 17.86±0.23 mg/g by the end of stage IV. Kumar *et al.*[11] suggested that cholesterol may be used as a substrate in the synthesis of steroid hormones in penaeid shrimp species. Crustacean ovary is known to be a centre for synthesis of vertebratetype steroids such as progesterone and estrogen[1]. Shih & Liao[12] reported that ovaries of the soldier crab, *Mictyris brevidactylus* could convert [^{3}H]-cholesterol into hormonal compounds such as pregnenolone, 17α-hydroxypregnenolone and progesterone. A decline in the cholesterol level of ovary, as observed in the present study, may be indicative of its utilization as substrate in the synthesis of vertebrate- type sex steroids. Previous radio-immunoassay of steroid hormones in the ovaries in *E. asiatica* has shown accumulation of estradiol-17 β and progesterone during oocyte maturation[13]. Glycolipid content of ovary in the present study showed a sharp decrease from 4.6% in stage I to 2.5% in stage IV ovary, suggesting that these carbohydrate prosthetic groups are progressively utilized during vitellogenesis as an energy-yielding substrate. Glycolipids bound to Vg may also have additional role in the recognition of Vg receptors on the oocyte membrane[14]. However, free fatty acid (FFA) increased significantly as the ovarian stages advanced ($P<0.05$). In general, total lipid in the ovary showed a high initial value in stage I ovary followed by a decline in stage II, and then a sharp rise in the final stage (Table 1).

The results of the present study further support that hepatopancreas is the major source of lipid for Vg synthesis in *E. asiatica*.The total lipids showed an increase in the hepatopancreas during the intermolt stage within which, the ovarian development is completed. This rise in total lipid level is followed precisely by a drastic decline in the major storage lipid, the TG, in the hepatopancreas, in correlation to the advancing ovarian development. Wen *et al.*[15] also recorded a decline in TG levels of the hepatopancreas in parallel to TG accumulation in the ovary during maturation of the crab, *Eriocheir sinensis*.TG level in hepatopancreas showed a significant decrease (75.30±15.62-50.27±2.60 mg/g) from the first stage ovary to the last. FFA content of hepatopancreas decreased gradually against a corresponding increase in the ovary during maturation. Conversely, cholesterol

Table 1 —Major lipid components (mg/g) of hepatopancreas, hemolymph and ovary at different ovarian stages of *Emerita asiatica*

Lipid classes	Hepatopancreas			Hemolymph			Ovary		
	I	II/III	IV	I	II/III	IV	I	II/III	IV
Total lipid	101.38±9.92^a	125.77±8.35^a	160.71±15.93^b	12.74±5.77^a	17.20±1.00^a	20.16±2.52^a	199.68±0.32^a	160.30±0.05^b	178.69±26.67^{ab}
Triglyceride	75.30±15.62^a	57.15±2.35^{ab}	50.27±2.60^b	ND	ND	ND	74.72±17.03^a	82.48±1.63^a	89.40±10.78^a
Cholesterol	11.74±0.16^a	12.69±0.11^b	13.97±0.56^c	0.39±0.18^a	0.41±0.04^a	0.65±0.09^a	20.49±0.63^a	19.72±0.2^a	17.86±0.23^b
Phospholipid	0.95±0.05^a	0.52±0.02^b	0.70±0.23^{ab}	0.05±0.02^a	0.05±0.01^a	0.03±0.01^a	0.94±0.01^a	0.95±0.07^a	1.18±0.18^a
Glycolipid	2.84±0.29^a	2.99±0.10^{ab}	3.76±0.55^b	0.46±0.03^a	1.42±0.11^b	0.75±0.09^c	9.18±4.49^a	4.60±0.03^a	4.44±0.19^a
FFA	6.75±0.04^a	4.78±0.02^{bc}	4.44±0.65^c	2.76±0.36^a	2.05±0.08^{bc}	1.74±0.24^c	2.92±0.9^a	3.89±0.09^a	6.78±1.34^b

[Values are mean ± S.D. (n=3, $P<0.05$). *ND, Not detectable]

levels in hepatopancreas rose steadily from first stage ovary (11.74±0.16 mg/g) to 13.97±0.56 mg/g in the fourth stage (P<0.05). Glycolipid content of hepatopancreas also increased significantly as the ovarian development proceeded. The apparent mobilization of hepatopancreatic carbohydrates to the ovary during vitellogenesis is reported in the penaeid shrimps *Fenneropenaeus merguiensis* and *F. penicillatus*[16]. In *E. asiatica*, hepatopancreatic glycolipids are conjugated to the Vg for their transport to the ovary, and both glucose (monoglycosylceramides) and galactose (diglycosylceramide)-containing glycolipids are bound to the major lipovitellin[17]. PL level in hepatopancreas was generally low, throughout ovarian maturation, although showing a slight fluctuation between ovarian stages. In the hemolymph, all lipid classes analysed were relatively low in concentration during different stages of ovarian development, notwithstanding a gradual increase of total lipids in the ovary. Table 2 summarises the changes in the fatty acid profile in the ovary and hepatopancreas during different stages of ovarian development. It may be noted that there is a reciprocal relationship in the changes of key fatty acid groups both in the ovary and hepatopancreas.

The fatty acid composition at different stages of vitellogenesis in the hepatopancreas and ovary of *E. asiatica* is given in Table 2. The results showed 22 fatty acids, among which, palmitic (16:0), palmitoleic (16:1n7) and eicosapentaenoic (20:5n3) acids were dominant. Saturated fatty acids (SFA) were the main groups, whereas PUFA and monounsaturated fatty acids (MUFA) formed the second and third group, respectively. The SFA content in the hepatopancreas ranged from 45.7 to 48.8%, the most predominant of which was C16:0 (Palmitic acid), ranging in concentration from 15.3 to 8.4%. SFA are nonessential fatty acids, which can be synthesized *de novo* or obtained by desaturation of MUFA and PUFA[18]. The MUFA content in the hepatopancreas ranged from 26.2 to 22.7%, with the most predominant, C16:1 (Palmitoleic acid), declining from 11.6 to 6.8%. The PUFA content in the hepatopancreas ranged from 25.0 to 31.2%, with the most predominant, C20:5n3 (Eicosapentaenoic acid), showing a decline from 9.8 to 5.6% during vitellogenesis. The present study also

Table 2 — Fatty acid composition (mg/g of total FA) at different stages of vitellogenesis in the hepatopancreas and ovary of the female *Emerita asiatica*

	Ovary			Hepatopancreas		
FA	Stage I	Stage II	Stage III/IV	Stage I	Stage II	Stage III/IV
C14:0	2.03±0.05^{a}	2.44±0.00^{b}	4.78±0.00^{c}	5.86±0.00^{a}	5.14±0.00^{b}	5.96±0.01^{c}
C15:0	0.33±0.01^{a}	0.50±0.01^{b}	0.61±0.00^{c}	1.10±0.01^{a}	0.92±0.01^{a}	0.51±0.01^{b}
C16:0	7.27±0.00^{a}	10.72±0.03^{b}	11.35±0.09^{c}	15.39±0.02^{a}	14.42±0.02^{b}	8.43±0.02^{c}
C17:0	0.37±0.01^{a}	0.53±0.00^{a}	0.91±0.19^{b}	0.50±0.02^{a}	0.59±0.02^{a}	0.37±0.04^{b}
C18:0	4.21±0.05^{a}	5.77±0.01^{b}	7.33±0.04^{c}	4.01±0.02^{a}	3.87±0.01^{b}	3.76±0.08^{b}
C20:0	0.08±0.01^{a}	0.20±0.01^{b}	0.26±0.00^{c}	0.32±0.03^{a}	0.30±0.02^{a}	0.20±0.01^{b}
ΣSFA	14.29±0.12^{a}	20.16±0.05^{b}	25.23±0.32^{c}	27.20±0.09^{a}	25.39±0.06^{b}	19.24±0.12^{c}
C14:1	0.32±0.02^{a}	0.64±0.01^{b}	0.31±0.03^{a}	0.46±0.01^{a}	0.40±0.01^{a}	0.63±0.05^{b}
C15:1	0.06±0.01^{a}	0.26±0.01^{b}	0.12±0.00^{c}	0.17±0.03^{a}	0.14±0.01^{ab}	0.20±0.02^{ab}
C16:1	5.27±0.02^{a}	8.76±0.01^{b}	7.73±0.20^{c}	11.76±0.01^{a}	10.45±0.02^{b}	6.77±0.00^{c}
C17:1	0.11±0.00^{a}	0.23±0.01^{b}	0.27±0.02^{c}	0.71±0.00^{a}	0.65±0.02^{b}	0.37±0.01^{c}
C18:1n9c	1.69±0.05^{a}	2.65±0.01^{b}	4.54±0.02^{c}	2.08±0.04^{a}	1.68±0.01^{b}	1.32±0.01^{c}
C20:1n9	0.09±0.00^{a}	0.22±0.01^{b}	0.17±0.00^{c}	0.13±0.00^{a}	0.16±0.01^{b}	0.12±0.01^{a}
C24:1n9	0.14±0.05^{a}	0.14±0.01^{a}	0.23±0.01^{b}	0.27±0.00^{a}	0.15±0.01^{b}	0.10±0.02^{c}
ΣMUFA	7.67±0.12^{a}	12.90±0.07^{b}	13.39±0.26^{c}	15.59±0.10^{a}	13.62±0.07^{b}	9.50±0.11^{c}
C18:2n6t	0.05±0.00^{a}	0.14±0.02^{b}	0.25±0.01^{c}	0.11±0.02^{a}	0.13±0.01^{a}	0.10±0.01^{a}
C18:2n6c	0.54±0.00^{a}	0.89±0.02^{b}	0.96±0.00^{c}	0.83±0.02^{a}	0.72±0.01^{b}	0.45±0.01^{c}
C18:3n6	0.05±0.00^{a}	0.26±0.01^{b}	0.34±0.00^{c}	0.32±0.01^{a}	0.34±0.00^{a}	0.20±0.01^{b}
C20:2	0.21±0.01^{a}	0.45±0.01^{b}	0.28±0.00^{c}	0.58±0.00^{a}	0.29±0.01^{a}	0.08±0.00^{b}
C20:3n6	0.09±0.01^{a}	0.19±0.02^{b}	0.46±0.03^{c}	0.31±0.05^{a}	0.21±0.03^{a}	0.48±0.03^{b}
C20:4n6	1.37±0.00^{a}	1.26±0.00^{b}	7.55±0.01^{c}	1.58±0.03^{a}	1.09±0.01^{b}	5.04±0.01^{c}
C22:2	0.10±0.01^{a}	0.32±0.05^{b}	0.31±0.02^{b}	0.41±0.00^{a}	0.31±0.01^{b}	0.11±0.01^{c}
C20:5n3	4.92±0.01^{a}	6.69±0.04^{b}	10.11±0.07^{c}	9.76±0.12^{a}	7.59±0.11^{b}	5.56±0.00^{c}
C22:6n3	2.29±0.03^{a}	3.16±0.03^{b}	3.14±0.02^{b}	2.79±0.04^{a}	2.07±0.03^{b}	1.00±0.01^{c}
ΣPUFA	9.62±0.07^{a}	13.37±0.12^{b}	23.41±0.15^{c}	16.68±0.24^{a}	13.02±0.17^{b}	13.05±0.05^{b}
ΣFFA	31.58±0.32^{a}	46.42±0.25^{b}	62.02±0.72^{c}	59.47±0.43^{a}	52.04±0.30^{b}	41.79±0.29^{c}

[*Values are means of triplicate samples ± SD. Within the same row, values with different superscript letters are significantly different (P<0.05); values with the same superscript letters are not significantly different (P>0.05). MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid]

revealed that ovarian lipids contain higher proportions of arachidonic acid (AA) than in hepatopancreatic lipids. AA is an important precursor of prostaglandins, and it is likely that part of (n-3) and (n-6) PUFA may be used as precursors for synthesis of these hormonal compounds[15]. Prostaglandins, synthesized from specific PUFA of membrane-bound phospholipids, have important physiological functions such as regulation of ion flux, temperature regulation, oocyte maturation, and control of larval hatching in marine crustaceans[19].

While the composition of major fatty acids in the ovary was similar to that in the hepatopancreas, the proportional amount of each fatty acid in the ovary varied between stages. The values of SFAs in the ovaries decreased significantly from 45.3 to 40.7% during maturation. The increasing proportions of SFA both in hepatopancreas and ovary, concurrent to a decrease in total lipid content in hepatopancreas imply mobilization of free fatty acids from hepatopancreatic reserves.In contrast, the MUFAs showed an increase from 24.3 to 27.8% in stage II, followed by a decrease to 21.6% in the final stage of vitellogenesis. The PUFA content, however, showed a steady increase from 30.5 to 37.7%, in the ovary during maturation. PUFA are essential nutrients, serving important functions in gonadal maturation of crustaceans[15]. The absence of PUFA in the maturation diet of the mud crab, *Scylla serrata*, has been shown to affect adversely the reproduction performance and fecundity[20].In particular, EPA and DHA promote fertilization and egg hatching[21]. Crustaceans have limited capability to synthesize DHA and EPA *de novo*, by virtue of their inability to convert 18:2n6 and 18:3n3 into C:20 and C:22 HUFA[22]. A diet deprived of adequate amount of lipid substrates has been shown to result in poor reproductive performance such as delayed spawning, low hatching rate, and reduced rate of larval survival in shrimps[23]. Microalgae are the generators and main suppliers of PUFA (linoleic and α-linolenic acid) in the marine ecosystem[24]. Since *Emerita* feeds directly on microalgae, the presynthesized fatty acid in the microalgae are readily available for assimilation and absorption into hepatopancreas of these marine crabs. Within the hepatopancreas, further enzymatic catalysis results in the formation of shorter and longer essential fatty acid[25].

HPLC separation of carotenoid pigments from the mature ovary and hepatopancreas of *E. asiatica* gave three major peaks in addition to several minor peaks (Fig. 1). The first major peak has a retention time of 3.616 min corresponding to that of β-carotene, the second at 4.175 min represents canthaxanthin, and the third at 14.164 min is astaxanthin. Quantitative changes of the three carotenoids in the ovary during maturation from stage I to IV is given in Fig. 2. β-carotene level in

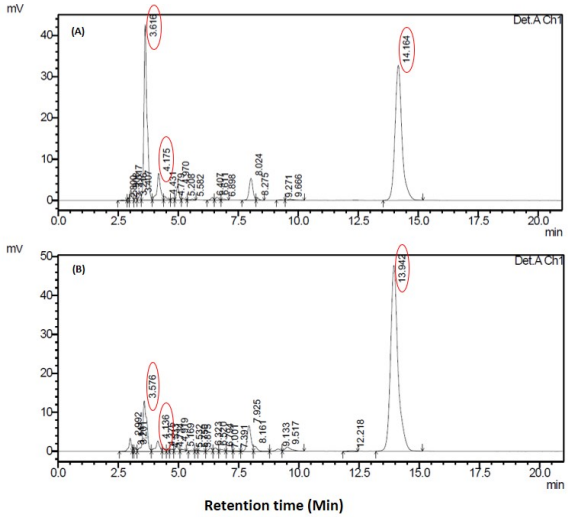

Fig. 1 — HPLC (at 450 nm) of carotenoids extracted from (A) ovary; and (B) hepatopancreas of stage I ovary

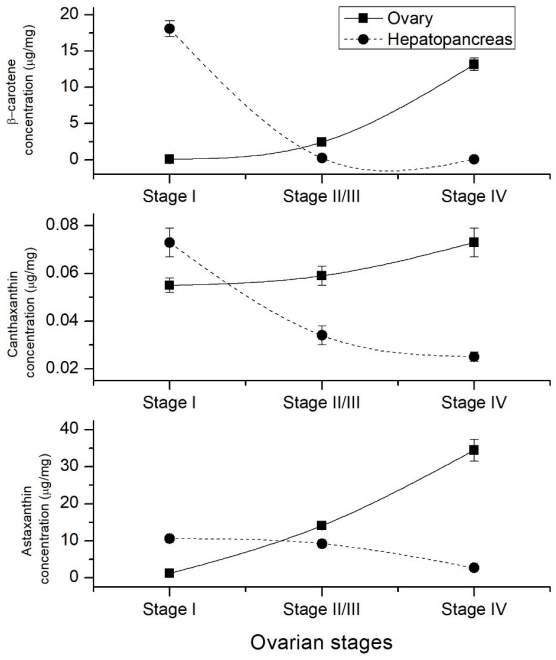

Fig. 2 — Distribution of different carotenoids (β-carotene, canthaxanthin and astaxanthin) in ovary and hepatopancreas of *Emerita asiatica.* [Values are mean ± S.D. (n=3, P<0.05)]

the ovary increased significantly from 0.0588±0.006 µg/mg in stage I to 13.153±0.87 µg/mg in stage IV. Relatively, the canthaxanthin level was very low, but the increase from early stage to late stage was significant. Astaxanthin level also rose steadily from 1.189±0.158 µg/mg to 34.440±2.919 µg/mg during ovarian maturation. The ovarian build-up of carotenoids during maturation has been reported in several crustaceans such as *Litopenaeus vannamei*[26] and *Metapenaeus Monoceros*[27]. Berticat *et al*.[28] suggested that carotenoids linked to vitellin molecules may protect the developing ovaries and embryos from visible wavelengths of light or over-radiation, as shown in the crayfish, *Astacus leptodactylus.* Carotenoids could also act as an antioxidant to protect the developing eggs from peroxidative damage by free radicals[29].

The predominant peak in the hepatopancreas is that of β-carotene, which showed a significant decline from 18.076±1.11 to 0.074±0.006 µg/mg as the maturation of ovary proceeded. A similar decrease of astaxanthin in the hepatopancreas was also recorded from stage I to IV (10.571±0.932 to 2.694±0.245 µg/mg), indicating an inverse relationship with the ovarian carotenoid changes. The intermediary compound, canthaxanthin also showed a decline in the hepatopancreas during ovarian development (0.073±0.006 to 0.025±0.002µg/mg). Castillo *et al*.[30]opined that hepatopancreas plays a major role in the absorption of carotenoids from digested food. In addition, Tantikitti *et al*.[31]found evidence that the decline in the carotenoids of hepatopancreas coincided with a corresponding increase in the ovary. The present study is in agreement with this observation, wherein the carotenoid content of the hepatopancreas was found to decrease, parallel to ovarian maturation. Obviously, there is an initial accumulation of carotenoids in the hepatopancreas during early vitellogenesis, followed by a gradual decline, indicating their mobilization to the ovary. Furthermore, the occurrence of various carotenoid pigments present within the ovary may suggest a possible interconversion of Vg-bound carotenoids into various other forms[32]. The changes found in the intermediary compound canthaxanthin adduce further evidence for this contention.

Astaxanthin is an important carotenoid accumulated within the ovary. Gilchrist & Lee[6] documented that astaxanthin is the predominant pigment in the eggs of *E. analoga* amounting to 45% of total carotenoids. The radiolabelled experiment by Gilchrist & Lee[6]have clearly demonstrated that *E. analoga* metabolize astaxanthin from ingested β-carotene. The fluctuations found in the levels of β carotene and astaxanthin, along with the intermediary compound canthaxanthin, may indicate that similar metabolic conversions could occur in *E. asiatica* both within the hepatopancreas and ovary.

Conclusion

Qualitative and quantitative analyses of various classes of lipids and carotenoid pigments in the hepatopancreas and ovary in the anomuran crab *Emerita asiatica* are reported in the present study. This analysis provides evidence that hepatopancreas synthesizes various fatty acids and the carotenoids and transfer them to the ovary by binding to the vitellogenin molecule. Whereas conjugated lipids like glycolipids are utilized as energy substrates, cholesterol serves as precursors for steroid hormone synthesis within the ovary. Furthermore, carotenoid pigments undergo metabolic conversions in the ovary after their uptake from the hepatopancreas.

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Conflict of Interest

Authors declare no competing interests.

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