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# Biochemical composition of the hemolymph, hepatopancreas, ovary, and muscle during ovarian maturation in the penaeid shrimps *Fenneropenaeus merguiensis* and *F. penicillatus* (Crustacea: Decapoda)

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**Abstract:** The present study quantifies the concentrations of proteins, carbohydrates, and lipids in the hemolymph, ovary, hepatopancreas, and muscle of *Fenneropenaeus merguiensis* and *F. penicillatus* during ovarian maturation. Proteins were the major constituent in the hemolymph and ovaries of the 2 species, while lipids were the major constituent in the hepatopancreas. The protein, carbohydrate, and lipid contents in the ovaries of both species increased as ovaries matured. The increase in the protein, carbohydrate, and lipid contents in the ovaries during maturation indicates that the ovaries utilize these constituents to mature. Concentrations of lipids in the hepatopancreas decreased with ovarian maturation in both species. It appears that the lipid demands of ovarian maturation are met by the hepatopancreas reserves, as lipid decrease in the hepatopancreas coincided with an increase in fully mature ovaries. The increase of protein, lipid, and carbohydrate contents in the hemolymph with ovarian maturation in both species shows that the hemolymph serves as a vehicle for the mobilization of organic reserves. Proteins, carbohydrates, and lipids in the muscles of the 2 species did not seem to be related to ovarian maturation. In the present study, both the gonadosomatic index and the hepatopancreatic index also increased with ovarian maturation in *F. merguiensis* and *F. penicillatus*.

**Key words:** Penaeid shrimps, ovarian maturation, proximate composition

## 1. Introduction

Information on biochemical changes and metabolic processes that occur during maturation are important for understanding crustacean reproduction. Therefore, a number of crustacean species have been examined for biochemical changes in the gonads, hepatopancreas, and muscle during maturation and molting (Pillay and Nair, 1973; Gehring, 1974; Kulkarni and Nagabhushanam, 1979; Read and Caulton, 1980; Castille and Lawrence, 1989; Rosa and Nunes, 2002; Hasek and Felder, 2005).

Two species, *Fenneropenaeus merguiensis* and *F. penicillatus*, are abundant in the shrimp catches of Pakistan, in the northern Arabian Sea (Zupanovic, 1971; van Zalinge et al., 1987), but over the past decade overfishing has caused tremendous stress on their stocks, which is evident from the decline in shrimp catches despite the increase in the number of fishing trawlers (Marine Fisheries Department, 2006). This decline can be alleviated by the development of shrimp farming in the country. The above-mentioned 2 species are among the species cultivated in various parts of the world (Qingbo et al., 1988; Chen et al., 1998; Hoang, 2001).

The establishment of the aquaculture industry has led to an increased interest in studying ovarian development and maturity, particularly in penaeid shrimps. Gonadal maturation in female shrimps can be evaluated visually on the basis of ovary size and color (Cummings, 1961; Brown and Patlan, 1974) or the examination of histological changes in the ovaries (Yano, 1988; Tan-Fermin and Pudadera, 1989; Ayub and Ahmed, 2002a). Maturation can also be evaluated on the basis of biochemical changes in the gonads, which are sites of intensive biochemical synthesis during gametogenesis. Increases in the carbohydrate, protein, and lipid contents of the ovaries during maturation have been reported in the shrimps *Metapenaeus affinis* (Pillay and Nair, 1973), *Penaeus duorarum* (Gehring, 1974), *Parapenaeopsis hardwickii* (Kulkarni and Nagabhushanam, 1979), *Penaeus indicus* (Read and Caulton, 1980), *Penaeus aztecus*, and *P. setiferus*

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(Castille and Lawrence, 1989). Ovarian maturation in decapods also requires neuropeptides, ecdysone, and methyl farnesoate, which are synthesized in the body through nutrients from the diet (Nagaraju, 2011). A number of neuropeptide hormones, such as vitellogenesis-inhibiting hormone (VIH), gonad-stimulating factor (GSF), crustacean hyperglycemic hormone (CHH), and molt-inhibiting hormone (MIH), play an essential role in controlling gonad maturation in crustaceans. CHH is known to regulate not only reproduction but also glucose levels in various organs of crustaceans. Ecdysteroids have been considered as molting hormones, but recent studies indicate that they play a major role in regulating vitellogenesis, ovarian maturation, and protein synthesis in decapods (Brown et al., 2009). It has been reported that crustaceans are reproductively active when the titers of VIH and ecdysteroid are low and those of GSF and methyl farnesoate are high (Chang et al., 2001; Nagaraju, 2007).

In contrast to other biological and fishery information on *F. merguiensis* and *F. penicillatus* from Pakistan and the northern Arabian Sea (Zupanovic, 1971; van Zalinge et al., 1987; Ayub and Ahmed, 1992, 2002a, 2002b), there is a lack of data from Pakistan related to biochemical composition in relation to maturity (Nisa and Sultana, 2010). Vitellogenesis is an important physiological process associated with female reproduction, which is characterized by the synthesis and accumulation of yolk protein (vitellin) in the developing oocytes, which acts as a nutrient source for developing embryos (Charniaux-Cotton, 1985). Vitellin is synthesized from vitellogenin precursors, which, depending on the species, can be synthesized in the hepatopancreas, ovaries, or both (Okumura et al., 2007). It is transported to the ovaries through the hemolymph and, once inside the ovaries, it is cleaved, forming lipovitellin subunits (Oberdorster et al., 2000; Okumura et al., 2007). Vitellogenin and lipovitellin are complex lipo-glyco-caroteno-proteins (Oberdorster et al., 2000). The present study was undertaken to measure protein, lipid, and carbohydrate concentrations in the hemolymph, hepatopancreas, ovaries, and muscle of *F. merguiensis* and *F. penicillatus* females with the progress of maturation.

## 2. Materials and methods

Female shrimps of *F. merguiensis* and *F. penicillatus* were captured by fishermen operating their trawler in the vicinity of Karachi, Pakistan. A total of 108 *F. merguiensis* and 71 *F. penicillatus* females were collected and transported to the laboratory alive, kept in well-aerated containers. It took 10 min to reach the laboratory, where the shrimps were transferred to 4 well-aerated circular tanks (diameter: 3.6 m, depth: 0.8 m, capacity: 8 $m^3$) at room temperature (26 °C). Immediately from each shrimp, the hemolymph (0.09 mL) was collected through the pericardial cavity and mixed with 10% EDTA (0.01 mL). The hemolymph was immediately centrifuged at 12,000 × *g* for 20 min, and supernatants were stored at –20 °C until further analysis. Shrimps were sacrificed and frozen. Each shrimp was measured for total length (cm) and weight (g) and then dissected on ice, and a 1.27-cm piece of tail muscle and the whole hepatopancreas and ovaries were removed. Females were classified as immature, maturing, and fully mature on the basis of the color of the ovaries, as per the criteria of Ayub and Ahmed (2002a). The ovary and hepatopancreas of each shrimp were weighed to calculate the gonadosomatic index (GSI) and hepatopancreatic index (HPI). The GSI was equal to the ovary weight divided by the total body weight multiplied by 100 (Giese, 1966). HPI for females was equal to hepatopancreas weight divided by total body weight minus ovary weight multiplied by 100 (Clarke, 1977). Tissue samples were placed in sample vials and stored at –20 °C until required.

### 2.1. Biochemical analysis

The total protein was estimated as per the Folin–Ciocalteu method of Lowry et al. (1951) with bovine serum albumin (BSA) as the standard. One gram of wet tissue of the ovary, hepatopancreas, and muscle was homogenized in a homogenizer (Model Polytron PT-MR 2100, Kinematic AG, Switzerland) with 10 mL of 0.1 M phosphate buffer. Next, 1 mL of tissue homogenate and 1 mL of 0.1 N NaOH were taken and kept for 30 min at room temperature. Subsequently, 8 mL of distilled water was added and centrifuged (Model 80-2, Seico, Pakistan) at 4000 rpm for 30 min. Only 0.1 mL of supernatant was taken, and 0.9 mL of distilled water was added to make up a volume of 1 mL. Next, 5 mL of alkaline reagent (2 g $Na_2CO_3$ in 0.1 N NaOH, 4% Na-K tartrate, and 2% $CuSO_4$, 200:1:1) was added and left for 30 min at room temperature. Finally, 0.5 mL of Folin phenol reagent was added and left for 40 to 45 min at room temperature. The color intensity was measured at 750 nm against a reagent blank on a spectrophotometer (Model 6306, Jenway, United Kingdom). The supernatant obtained was used for the estimation of carbohydrate content following the method of Dubois et al. (1956).

The lipids were quantitatively determined by the sulfo-phospho-vanillin method of Barnes and Blackstock (1973). For this, 1 g each of the wet tissues of the ovary, hepatopancreas, and muscle from each reproductive stage taken in clean dry test tubes was homogenized in 10 mL of chloroform and methanol mixture (2:1, v/v) and centrifuged at 4000 rpm for 20 min. After centrifugation, 0.5 mL of supernatant containing the lipids was taken in a test tube and evaporated in a warm water bath. The process of evaporation continued until dryness. The tube was left at room temperature for 3 min and, after the addition of 2 mL of concentrated $H_2SO_4$, it was covered with aluminum

foil and placed in boiling water for 10 min. The tube was then kept in cold water for 5 min. In another test tube, 0.1 mL of mixture was taken from the cool test tube, 5 mL of phospho-vanillin reagent was added, and the mixture was incubated for 15 min at 37 °C. The absorbance was read at 540 nm against the blank on a spectrophotometer (Model 6306, Jenway, United Kingdom). A calibration curve using cholesterol as a standard was constructed. Phospho-vanillin reagent was prepared by mixing 1.2 g of vanillin, 200 mL of distilled water, and 800 mL of orthophosphoric acid.

### 2.2. Statistical analyses

Biometric differences among maturation stages in *F. merguiensis* and *F. penicillatus* were analyzed by one-way ANOVA. Differences in the biochemical composition of the hemolymph, ovary, hepatopancreas, and muscle with species and maturation stages as factors were analyzed by 2-way ANOVA. If factors were significant, the data were rechecked with one-way ANOVA and a Tukey test (multiple comparison test) to see which factors were different. Regression analysis was used to determine the relationship between GSI and ovarian or hepatopancreatic biochemical constituents. Pearson correlations between GSI and ovarian or hepatopancreatic biochemical constituents were computed. SPSS 14.0 was used to analyze the data.

## 3. Results

### 3.1. Biometric data

Carapace length, total length, and body weight did not differ among the females of *F. merguiensis* and *F. penicillatus* in different maturation stages (Table). As ovarian maturation progressed in *F. merguiensis*, ovary weight and GSI increased significantly ($F = 67.28$, $df = 2$, $P < 0.001$; $F = 106.89$, $df = 2$, $P < 0.001$, respectively). The same result was found in *F. penicillatus*, where ovary weight and GSI increased significantly during ovarian maturation ($F = 49.74$, $df = 2$, $P < 0.001$; $F = 93.73$, $df = 2$, $P < 0.001$, respectively). Similarly, the hepatopancreatic weight and HPI were significantly different from one level of ovarian maturation to another in *F. merguiensis* ($F = 11.08$, $df = 2$, $P < 0.001$; $F = 28.17$, $df = 2$, $P < 0.001$, respectively) and *F. penicillatus* ($F = 20.87$, $df = 2$, $P < 0.001$; $F = 24.46$, $df = 2$, $P < 0.001$, respectively) (Table).

### 3.2. Biochemical composition of the hemolymph

The percentage of proteins in the hemolymph of *F. merguiensis* varied from 11.7% to 20.0%, that of carbohydrates from 0.02% to 0.04%, and that of lipids from 0.07% to 0.13%. In *F. penicillatus* the percentage of proteins in the hemolymph varied from 13.0% to 20.0%, that of carbohydrates from 0.02% to 0.04%, and that of lipids from 0.07% to 0.14%. Protein was the major constituent in the hemolymph of both species.

**Table.** Biometric mean ± standard deviation for females at different stages of ovarian maturation. CL = carapace length; TL = total body length; BW = body weight; GW = gonad weight; GSI = gonadosomatic index; HPW = hepatopancreas weight; HPI = hepatopancreatic index. Values in a row with different superscripts (a, b, c) are significantly different ($P < 0.05$). Values without a superscript are not significantly different.

| *F. merguiensis* | Immature (n = 25) | Maturing (n = 52) | Fully mature (n = 31) |
|---|---|---|---|
| CL (mm) | 57.5 ± 5.7 | 55.3 ± 5.7 | 55.5 ± 6.4 |
| TL (mm) | 152.8 ± 11.1 | 154.2 ±15.4 | 155.9 ± 16.3 |
| BW (g) | 32.6 ± 13.9 | 32.4 ± 11.2 | 34.5 ± 13.6 |
| GW (g) | 0.8 ± 0.2 [a] | 1.9 ± 0.7 [b] | 3.2 ± 1.1 [c] |
| GSI | 2.7 ± 0.7 [a] | 5.9 ± 1.8 [b] | 9.5 ± 2.1 [c] |
| HPW (g) | 0.7 ± 0.3 [a] | 0.8 ± 0.3 [b] | 1.0 ± 0.3 [c] |
| HPI | 2.1 ± 0.5 [a] | 2.8 ± 0.7 [b] | 3.5 ± 0.9 [c] |
| *F. penicillatus* | Immature (n = 28) | Maturing (n = 26) | Fully mature (n = 17) |
| CL (mm) | 58.3 ± 4.0 | 59.1 ± 4.8 | 61.7 ± 6.8 |
| TL (mm) | 160.9 ± 9.3 | 163.3 ± 14.3 | 170.3 ± 22.7 |
| BW (g) | 35.8 ± 5.6 | 36.2 ± 12.4 | 43.2 ± 17.8 |
| GW (g) | 1.1 ± 0.4 [a] | 2.4 ± 1.0 [b] | 4.2 ± 1.5 [c] |
| GSI | 3.2 ± 0.8 [a] | 6.7 ± 1.4 [b] | 9.7 ± 2.5 [c] |
| HPW (g) | 0.8 ± 0.2 [a] | 1.0 ± 0.3 [b] | 1.3 ± 0.4 [c] |
| HPI | 2.2 ± 0.4 [a] | 2.9 ± 0.6 [b] | 3.6 ± 1.0 [c] |

The concentrations of total proteins, carbohydrates, and lipids in the hemolymph showed no significant differences between the 2 species. The concentration of proteins and lipids in the hemolymph of *F. merguiensis* (Figure 1) and *F. penicillatus* (Figure 2) varied significantly ($P < 0.001$) with maturation in the ovaries, being lowest in immature shrimp (147.0 mg $mL^{-1}$ protein and 75 mg $dL^{-1}$ lipid in *F. merguiensis* and 143.0 mg $mL^{-1}$ protein and 71.4 mg $dL^{-1}$ lipid in *F. penicillatus*) and highest in fully mature shrimp (191.0 mg $mL^{-1}$ protein and 126 mg $dL^{-1}$ lipid in *F. merguiensis* and 188.0 mg $mL^{-1}$ protein and 129.0 mg $dL^{-1}$ lipid in *F. penicillatus*). The concentration of carbohydrates in the hemolymph was not different in immature (25.7 mg $dL^{-1}$) and maturing females (30.1 mg $dL^{-1}$) of *F. merguiensis*, but it was significantly different ($P < 0.05$) in fully mature females (36.2 mg $dL^{-1}$) (Figure 1). The concentration of

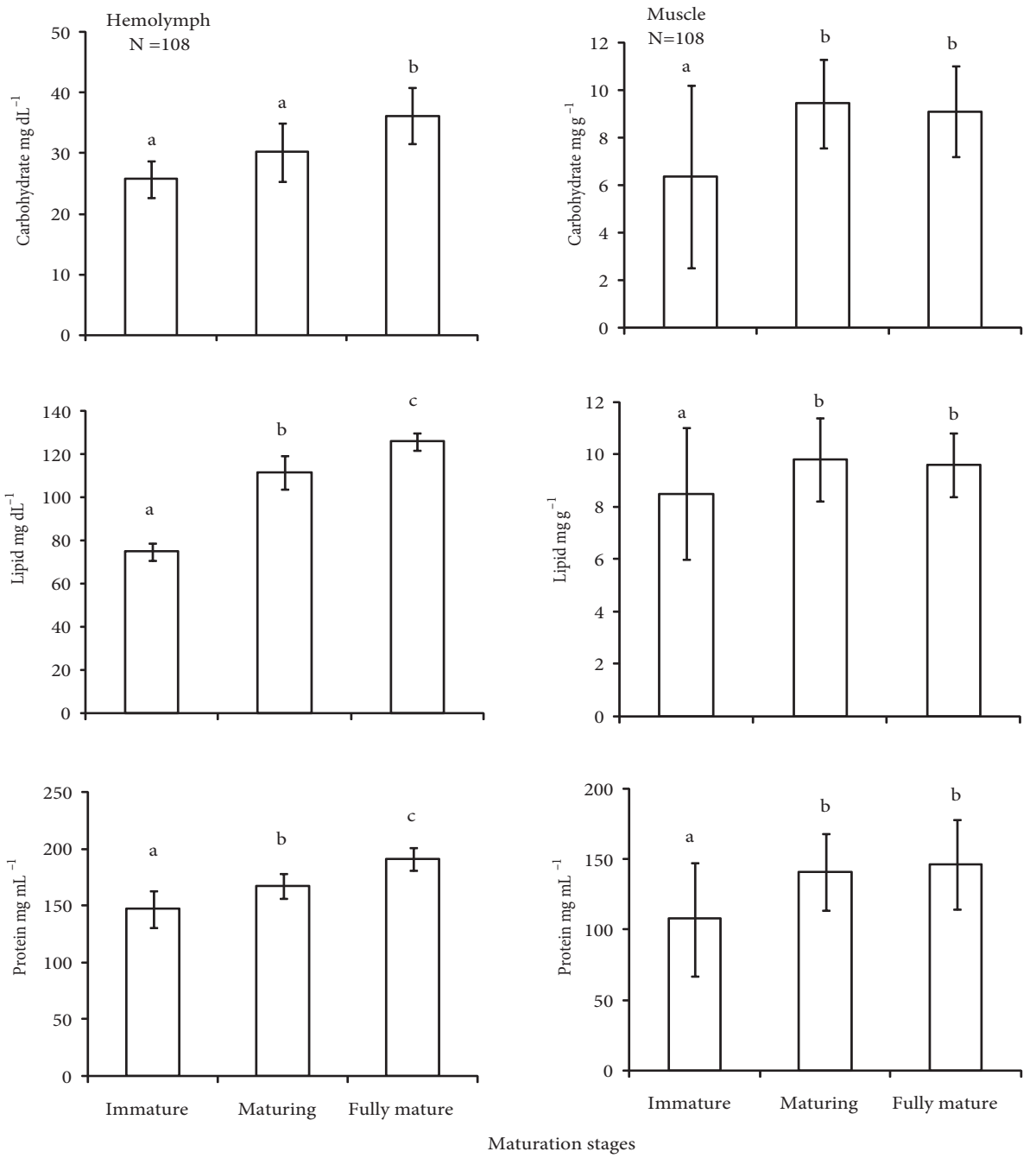


**Figure 1.** Mean ± standard deviation of proteins, lipids, and carbohydrates in the hemolymph and muscle of *F. merguiensis* in different stages of maturation. Means with different letters are significantly different at $P < 0.05$.

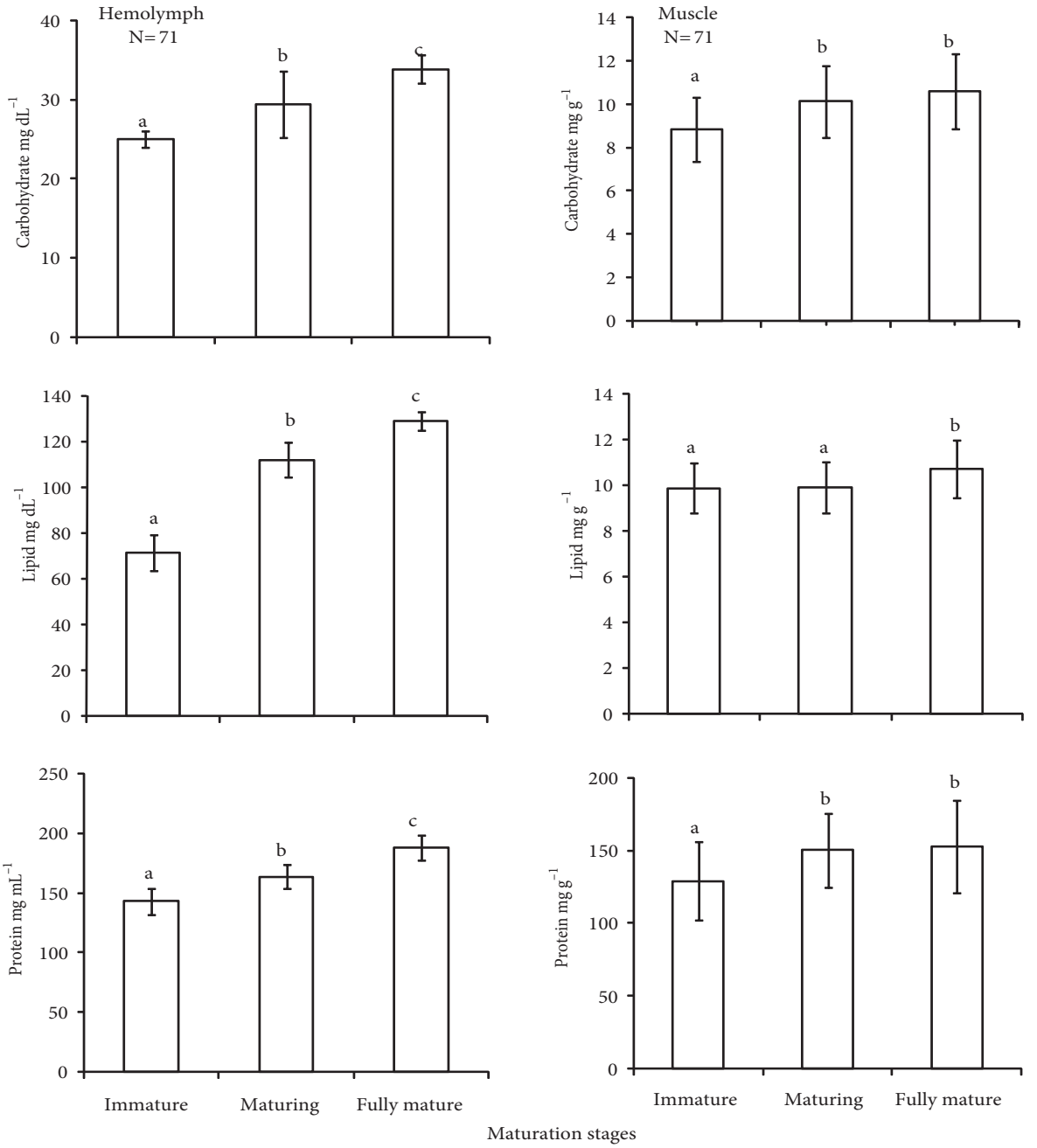


**Figure 2.** Mean ± standard deviation of proteins, lipids, and carbohydrates in the hemolymph and muscle of *F. penicillatus* in different stages of maturation. Means with different letters are significantly different at $P < 0.05$.

carbohydrates in the hemolymph of *F. penicillatus* varied significantly ($P < 0.001$) with maturation stages (Figure 2).

### 3.3. Biochemical composition of the ovary

The percentage of proteins in the ovarian tissue of *F. merguiensis* varied from 6.0% to 21.3%, that of carbohydrates from 0.3% to 2.7%, and that of lipids from 1.1% to 5.2%. In *F. penicillatus* the percentage of proteins varied from 8.9% to 22.5%, that of carbohydrates from 1.1% to 3.1%, and that of lipids from 1.2% to 6.0%. Protein was the major constituent in the ovary of the 2 species.

In the ovarian tissue there were significant differences in concentrations of proteins, lipids, and carbohydrates between *F. merguiensis* and *F. penicillatus*. Ovary protein, lipid, and carbohydrate concentrations increased significantly in ovary tissue as the ovary stage advanced in *F. merguiensis* (Figure 3). Similar significant increases

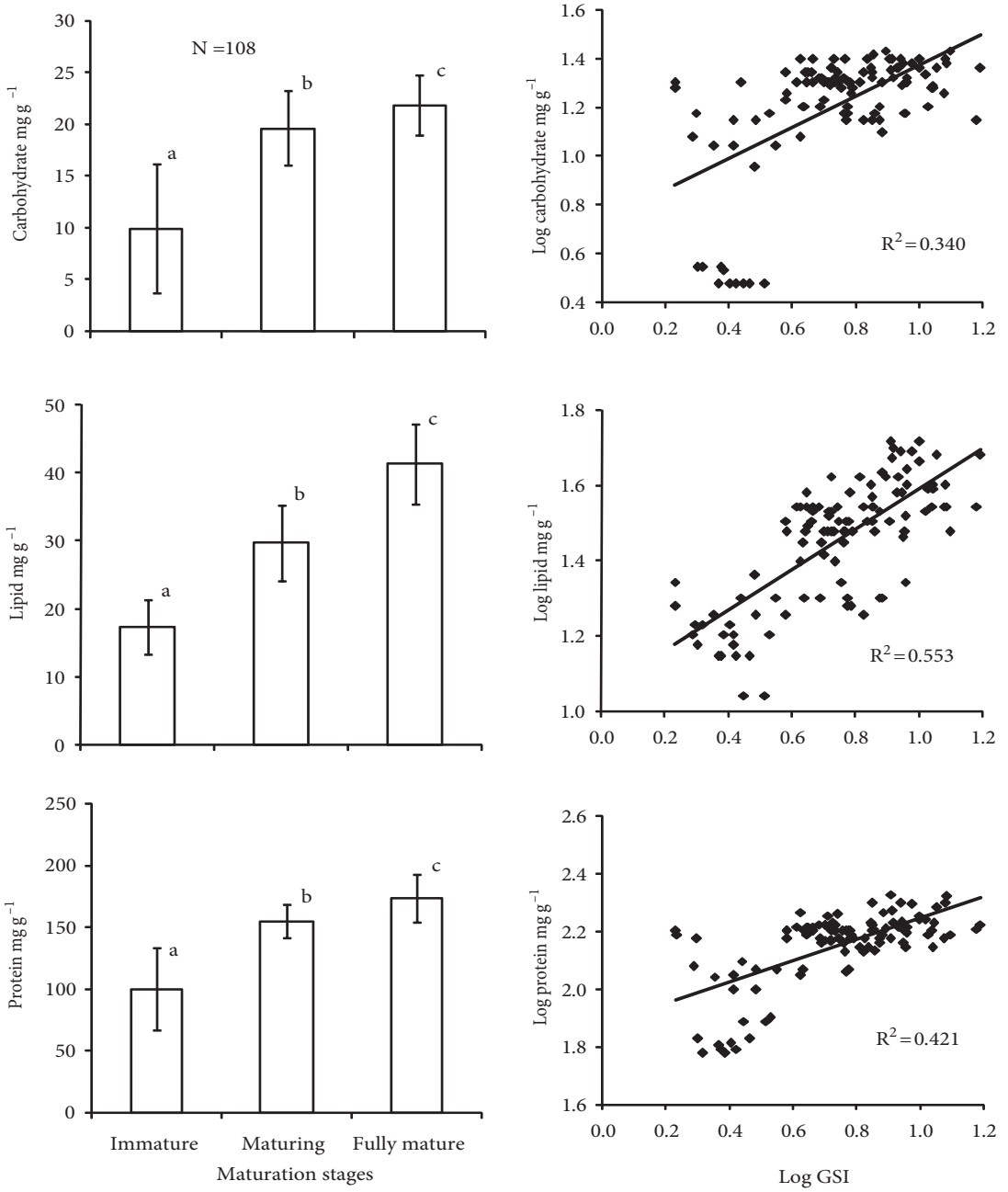


**Figure 3.** Mean ± standard deviation of proteins, lipids, and carbohydrates in the ovaries of *F. merguiensis* in different stages of maturation. Logarithmic ovary protein, lipid, and carbohydrate regressed against logarithmic GSI. Means with different letters are significantly different at $P < 0.05$.

in biochemical contents were observed in the ovary tissue of *F. penicillatus* with increase in maturation (Figure 4). The GSI in *F. merguiensis* showed a statistically significant (P = 0.01) positive correlation with ovary protein, lipid, and carbohydrate concentrations ($r^2 = 0.582$, $r^2 = 0.681$, and $r^2 = 0.5041$, respectively) (Figure 3). The GSI in *F. penicillatus* showed a statistically significant positive correlation with ovary protein ($r^2 = 0.654$, P = 0.01), lipid ($r^2 = 0.774$, P = 0.01), and carbohydrate ($r^2 = 0.748$, P = 0.01) concentrations (Figure 4).

### 3.4. Biochemical composition of hepatopancreas

In *F. merguiensis* the percentage of proteins in the hepatopancreas varied from 7.0% to 14.7%, that of carbohydrates from 0.4% to 3.8%, and that of lipids from 6.0% to 20.0%. In *F. penicillatus* the percentage of proteins in the hepatopancreas varied from 5.9% to 13.5%, that

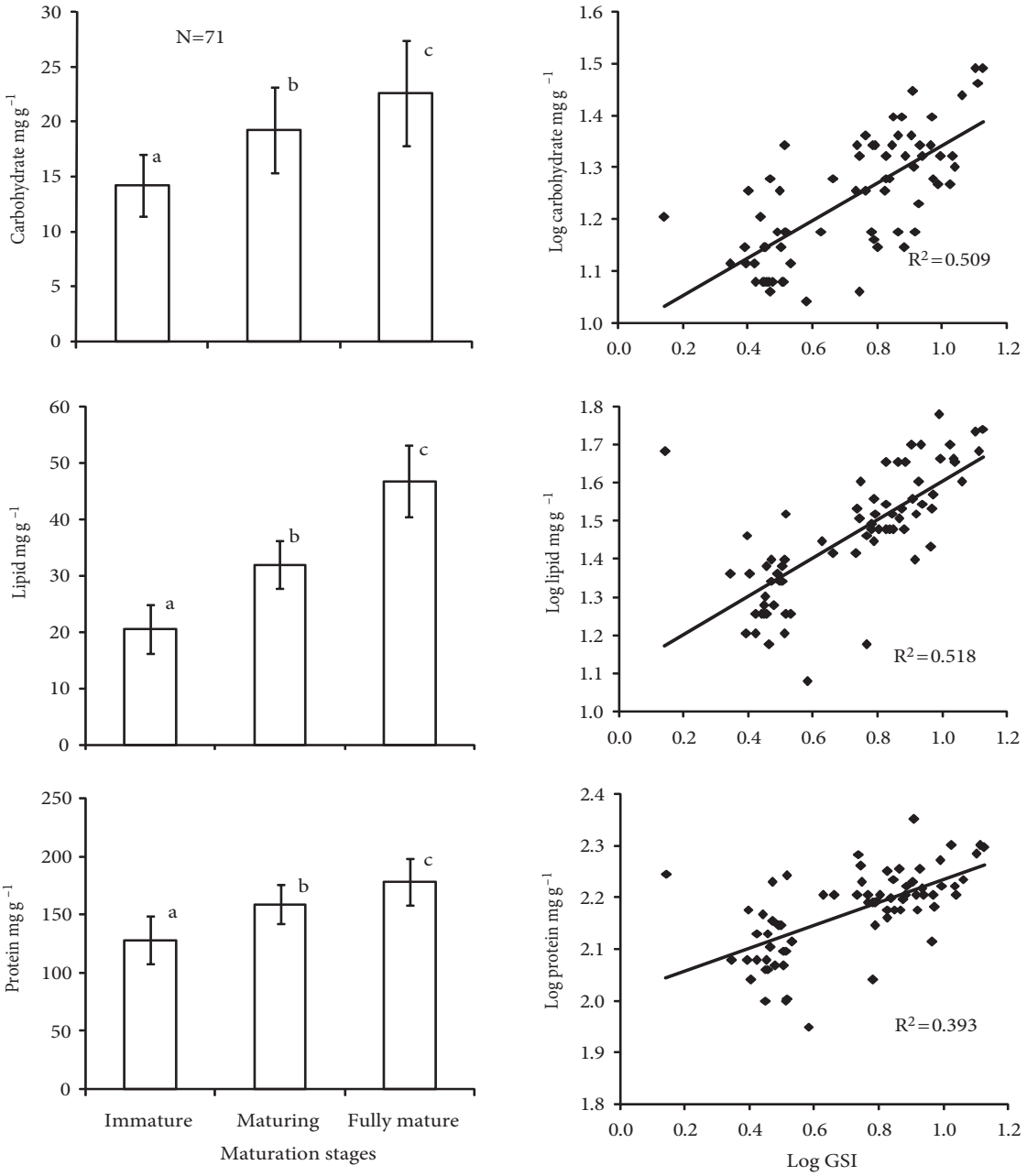


**Figure 4.** Mean ± standard deviation of proteins, lipids, and carbohydrates in the ovaries of *F. penicillatus* in different stages of maturation. Logarithmic ovary protein, lipid, and carbohydrate regressed against logarithmic GSI. Means with different letters are significantly different at $P < 0.05$.

of carbohydrates from 1.1% to 3.8%, and that of lipids from 8.5% to 18.0%. From these data, lipids are the major constituent in the hepatopancreas of the 2 species.

The concentrations of total proteins, carbohydrates, and lipids in the hepatopancreas showed significant differences between the 2 species. The concentrations of total proteins and carbohydrates in the hepatopancreas of *F. merguiensis* and *F. penicillatus* were found to increase with the progress of ovarian development (Figures 5 and 6, respectively). The concentrations of lipid in the hepatopancreas were highest in *F. merguiensis* (136.2 mg $g^{-1}$) (Figure 5) and *F. penicillatus* (141.9 mg $g^{-1}$) (Figure 6) with immature ovaries, and lowest in fully mature ovaries. The GSI in *F. merguiensis* showed a statistically significant positive correlation with hepatopancreas protein concentrations ($r^2 = 0.498$, $P = 0.01$) and carbohydrate concentrations ($r^2 = 0.426$, $P = 0.01$); however, the GSI of this species showed a statistically significant negative correlation with hepatopancreas lipid

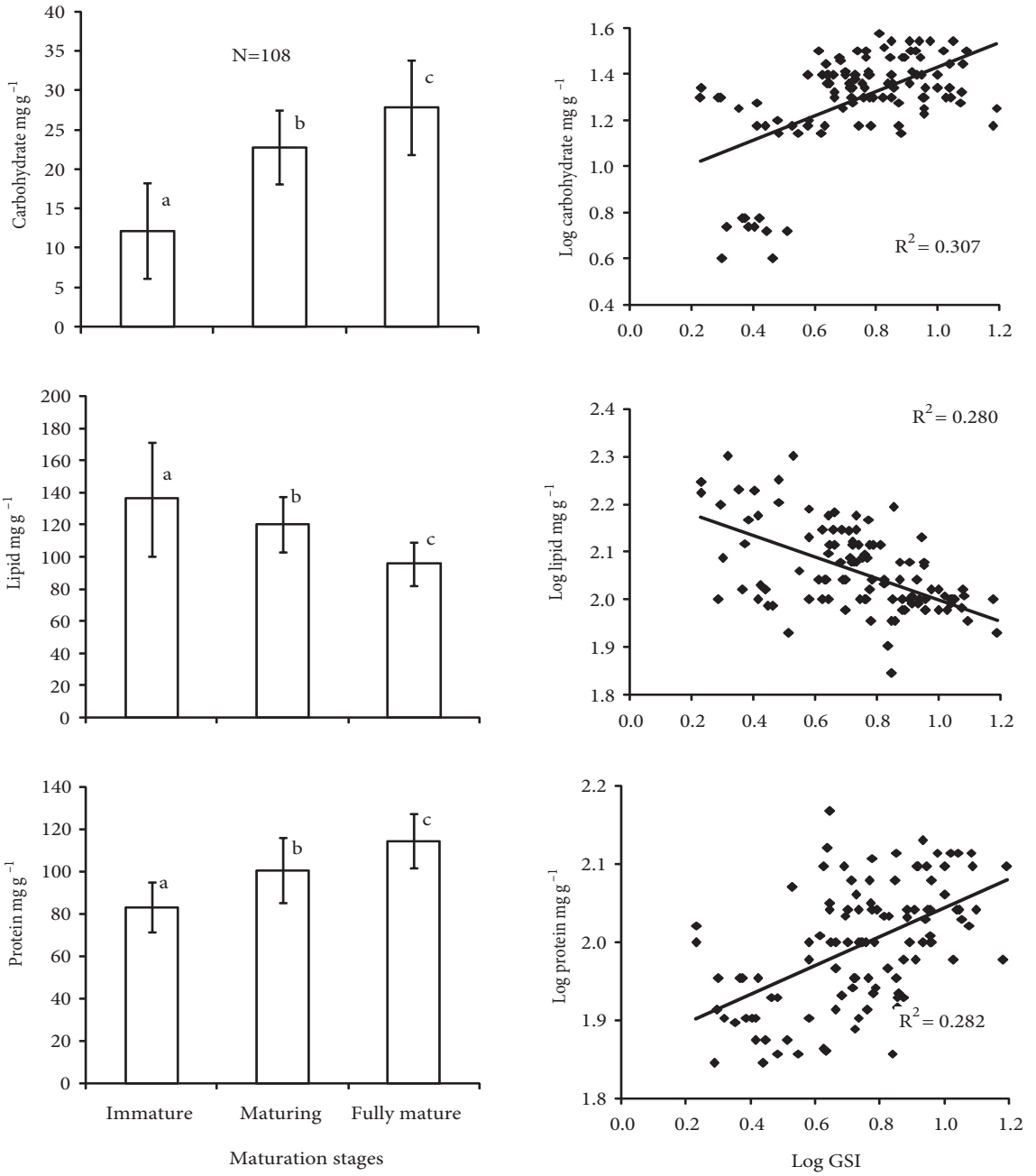


**Figure 5.** Mean ± standard deviation of proteins, lipids, and carbohydrates in the hepatopancreas of *F. merguiensis* in different stages of maturation. Logarithmic hepatopancreas protein, lipid, and carbohydrate regressed against logarithmic GSI. Means with different letters are significantly different at $P < 0.05$.

concentration ($r^2 = -0.520$, $P = 0.01$) (Figure 5). The GSI in *F. penicillatus* showed a statistically significant positive correlation with hepatopancreas protein concentrations ($r^2 = 0.662$, $P = 0.01$) and carbohydrate concentrations ($r^2 = 0.694$, $P = 0.01$), while the GSI showed a statistically significant negative correlation with hepatopancreas lipid concentration ($r^2 = -0.487$, $P = 0.01$) (Figure 6).

### 3.5. Biochemical composition of muscles

In *F. merguiensis*, the percentage of proteins in the muscle varied from 6.0% to 21.0%, that of carbohydrates from 0.2% to 1.4%, and that of lipids from 0.3% to 1.6%. In *F. penicillatus*, the percentage of proteins in the muscle varied from 9.8% to 21.0%, that of carbohydrates from 0.7% to 1.3%, and that of lipids from 0.8% to 1.2%. Protein was found to be the major constituent in the muscle.

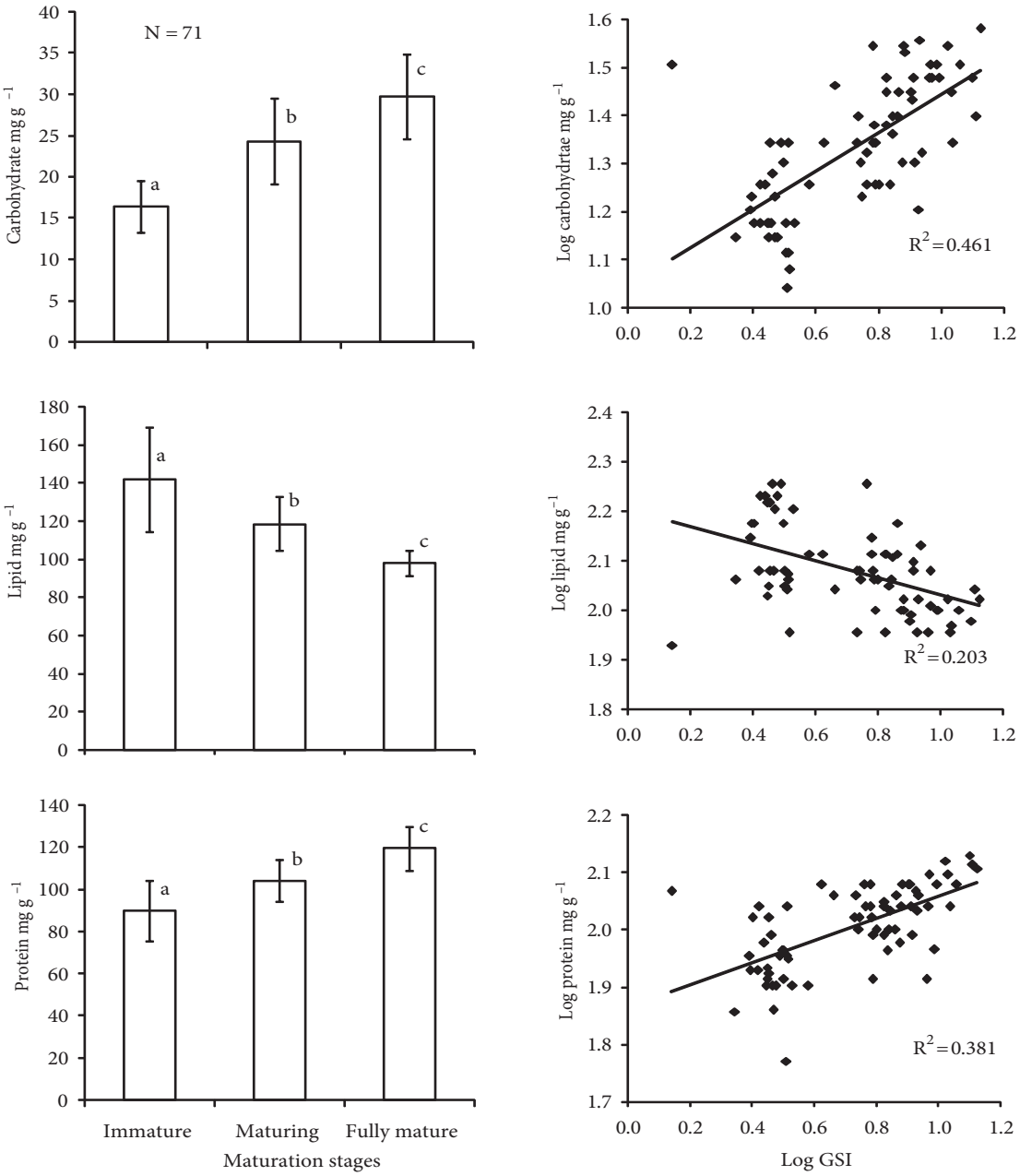


**Figure 6.** Mean ± standard deviation of proteins, lipids, and carbohydrates in the hepatopancreas of *F. penicillatus* in different stages of maturation. Logarithmic hepatopancreas protein, lipid, and carbohydrate regressed against logarithmic GSI. Means with different letters are significantly different at $P < 0.05$.

The concentrations of total proteins, carbohydrates, and lipids in the muscle showed significant differences between the 2 species. Muscle protein, lipid, and carbohydrate concentrations were lowest in immature females of *F. merguiensis* (Figure 1) and differed significantly ($P < 0.001$) from both maturing and fully mature females. The concentration of these constituents was not different between maturing and fully mature females of *F. merguiensis* (Figure 1). The concentration of proteins and carbohydrates in the muscle of *F. penicillatus* was not significantly different in maturing and fully mature females, but it was significantly different ($P < 0.001$) in immature females (Figure 2). Muscle lipid concentration was not different in immature and maturing females of *F. penicillatus*, but it was significantly different ($P < 0.05$) in fully mature females (Figure 2).

### 3.6. Comparison of proteins, carbohydrates, and lipids in the hepatopancreas, hemolymph, and ovary

The comparison of the concentrations of protein in the hepatopancreas, hemolymph, and ovary showed that the hemolymph had the highest concentrations of protein while the hepatopancreas had the lowest in immature, maturing, and fully mature females. The concentrations of carbohydrates were highest in the hemolymph and lowest in the ovaries in immature, maturing, and fully mature females; however, lipids were highest in the hepatopancreas in immature and maturing females, while in fully mature female lipids were comparatively higher in the hemolymph than in the hepatopancreas. This trend was found in both species, *F. merguiensis* and *F. penicillatus* (Figure 7).

## 4. Discussion

In the present study, the translucent, white, cream, and yellow ovaries of *P. merguiensis* and *P. penicillatus* were considered immature; greenish white or light green ovaries were considered maturing; and dark or dull green ovaries were considered fully mature, based on the study by Ayub and Ahmed (2002a). In their study, Ayub and Ahmed (2002a) reported that in *F. merguiensis* and *F. penicillatus*, translucent, white, cream, and yellow ovaries upon histological examination were revealed to be in an immature or spent stage; light green ovaries in a nearly ripe stage; and dark green ovaries in a fully ripe stage. The immature and spent females were grouped together, as Suneetha et al. (2009) reported that in the spent stage there is a decrease in the content of all body

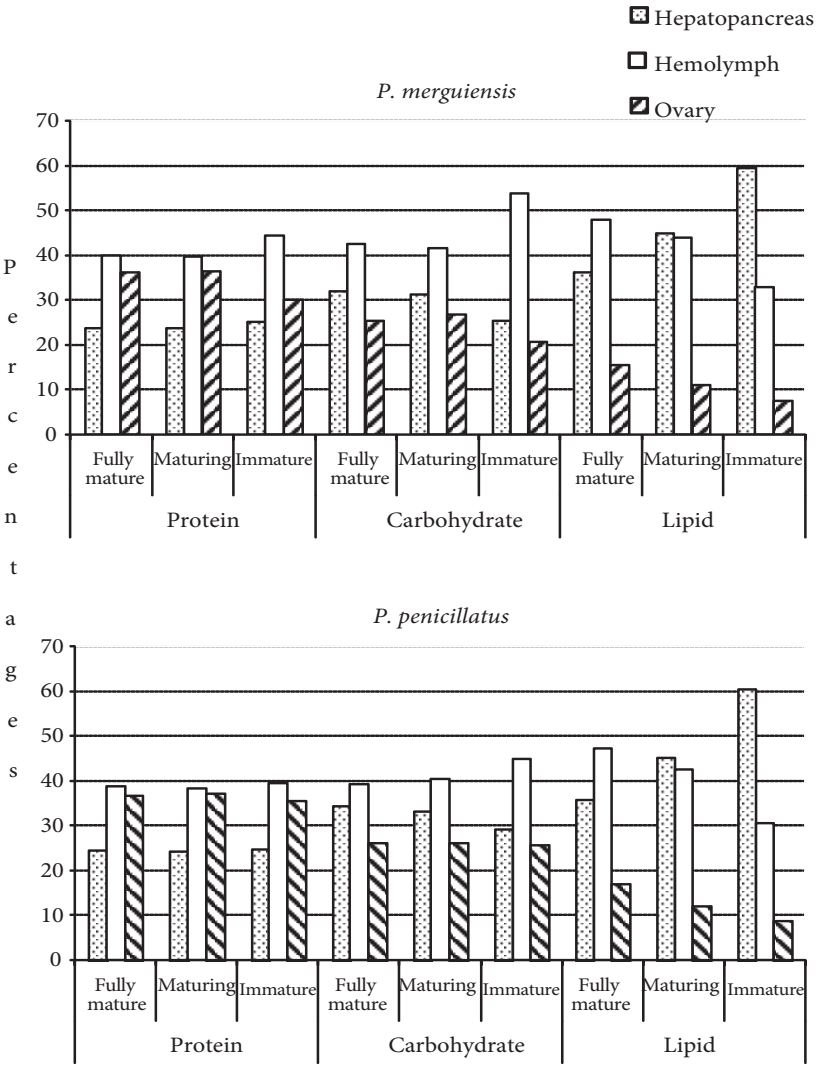


**Figure 7.** Comparison of protein, carbohydrate, and lipid in hepatopancreas, hemolymph, and ovary of immature, maturing, and fully mature females of two species.

constituents of *P. monodon*, similar to that at the onset of maturation. Similarly, Peixoto et al. (2003) concluded that the morphology and color of the ovaries were very similar in the immature and spent stage in female *F. paulensis*.

In the present study, both the GSI and HPI increased with ovarian maturation in *F. merguiensis* and *F. penicillatus* according to the data reported for *A. antennatus*, *P. longirostris*, and *N. norvegicus* (Rosa and Nunes, 2002). In the present study, though lipids decreased in the hepatopancreas of females with fully mature ovaries in both species, the HPI was observed to increase with increasing maturation. This may be accounted for by the fact that, although lipids are decreased, the concentrations of proteins and carbohydrates are increased, which may result in an increase in the weight of the hepatopancreas and thus an increase in HPI.

The protein, carbohydrate, and lipid contents in the ovaries of *F. merguiensis* and *F. penicillatus* were observed to increase with ovarian maturation. This result is similar to those of other studies, as increases in the organic constituents of the ovaries during maturation have been reported in the shrimps *Metapenaeus affinis* (Pillay and Nair, 1973), *Penaeus duorarum* (Gehring, 1974), *Penaeus indicus* (Read and Caulton, 1980), and *Parapenaeopsis hardwickii* (Kulkarni and Nagabhushanam, 1979). The lipoprotein present in the ovaries is termed lipovitellin, which is first synthesized as the precursor, vitellogenin, in the hepatopancreas, ovaries, or both, and then transported via hemolymph to the oocytes, where it is cleaved and accumulated, forming lipovitellin subunits (Oberdorster et al., 2000; Okumura et al., 2007). The increase in the protein, carbohydrate, and lipid contents in the ovaries during maturation indicates that the ovaries have absorbed these constituents for maturation.

In the present study, the concentrations of lipids in the hepatopancreas of *F. merguiensis* and *F. penicillatus* were highest in immature females and decreased with maturity, being lowest in fully mature females. The decrease in lipids in the hepatopancreas with maturity shows that vitellogenin is synthesized in the hepatopancreas and later transported to the ovaries such that the lipid contents are at maximum concentration in fully mature ovaries, suggesting that the reserves of lipids have been deposited in fully developed oocytes. Mak et al. (2005) suggested that vitellogenin is first synthesized in the hepatopancreas of the crab *Charybdis feriatus* and afterwards taken up by the ovary. Okumura et al. (2007) suggested that vitellogenin is synthesized in the hepatopancreas, ovaries, or both and then transported to the ovaries, where it is cleaved, forming lipovitellin subunits in the penaeid shrimp *P. japonicus*. The presence of increased levels of vitellogenin in the ovary and hepatopancreas during yolk accumulation has been reported in several penaeid shrimps (Avarre et al., 2003; Tiu et al., 2006; Phiriyangkul and Utarabhand, 2006; Raviv et al., 2006). Vitellogenin and lipovitellin are complex lipo-glyco-caroteno-proteins (Oberdorster et al., 2000). The present results are in accordance with those obtained in different penaeid shrimps (Khayat et al., 1994; Palacios et al., 2000; Vazquez Boucard et al., 2002) and crabs (Khan and Natarajan, 1980; Nagabhushanam and Farooqui, 1982; Mourente et al., 1994).

Unlike the present study, in which a decrease in hepatopancreas lipids was found with ovarian maturation, there are studies on some decapods in which no maturity-related decrease in HP lipids was observed (Castille and Lawrence, 1989; Cavalli et al., 2001; Rosa and Nunes, 2002). The authors of these studies reported that in such cases the lipid requirements of the developing ovary are dependent on the ingestion of dietary lipids rather than on HP reserves. However, Antunes et al. (2010), while studying seasonal variations in the biochemical composition of the ghost crab, *Ocypode quadrata*, reported that lipids seemed to be an important reserve of energy used during reproduction, in both males and females, whereas glycogen may be used during periods of intense activity or fasting.

In the present study, the protein content in the ovary and hepatopancreas increased significantly during maturation, which is in accordance with the findings of Tuck et al. (1997), who observed an increase in the protein content of the ovary and hepatopancreas during maturation in *N. norvegicus*, but not in accordance with those of Rosa and Nunes (2002), who observed no significant increase in protein in these tissues in the same species.

Carbohydrates showed significant increases in the ovary of *F. merguiensis* and *F. penicillatus* as maturation advanced, which contradicts the study by Rosa and Nunes (2002), who found that carbohydrates did not show significant variations throughout maturation in *N. norvegicus*, while Tuck et al. (1997) found that carbohydrates decreased with maturation in the same species. In the present study, the GSI of both species was correlated positively with ovary carbohydrate concentration, showing its possible role in the maturation of the ovaries.

Crustaceans digest and absorb lipids in their feed and transport them to the appropriate cells for utilization or storage. The main storage organ for lipids in shrimp is the hepatopancreas (Dall et al., 1990). Lipids are hydrophobic in nature and thus require a special vehicle for their transport through the aqueous hemolymph. For this purpose, in crustaceans, as in other animals, the lipids get associated with proteins, forming lipoproteins. Two different lipoproteins, lipoprotein I and very high density lipoprotein (VHDL), were isolated from the male hemolymphs of crustaceans, while in females, in addition to these, lipoprotein II was present, which seemed to be

vitellogenin (Komatsu et al., 1993). Garcia et al. (2002) also showed the presence of lipoproteins in crustacean serum in *Macrobrachium borellii*, and termed them HDL-1 and HDL-2. According to Garcia et al. (2002), HDL-1 was present in the plasma of both males and females, while HDL-2, also called vitellogenin, was only present in females and carried the yolk precursors from extraovarian synthesis sites to the oocyte. The presence of proteins, lipids, and carbohydrates in the hemolymph of *F. merguiensis* and *F. penicillatus* shows that vitellogenin has been released from the hepatopancreas or ovaries, or both, to be absorbed by the developing oocytes. The hemolymph serves as a special vehicle for the mobilization of organic reserves to the other tissues.

In the present study, proteins, carbohydrates, and lipids in the muscles of the 2 species do not seem to be related to the maturation stages. Protein was found to be the major constituent in the muscle of the shrimp. No differences in protein content between the 2 species were observed, although *F. penicillatus* had a higher carbohydrate and lipid content in the muscle than did *F. merguiensis*. On the whole, the proximate composition of the edible part of the 2 species was found to be different. The same difference in proximate composition has been reported in red shrimp and pink shrimp (Rosa and Nunes, 2003), and in black tiger shrimp and white shrimp (Sriket et al., 2007). The proximate compositions of shrimp muscles are dependent on factors such as species, growth stage, feed, and season (Sikorski et al., 1990; Karakoltsidis et al., 1995).

The present study shows that the concentrations of lipids in the hepatopancreas for both species were highest in immature females and decreased with maturity, being lowest in fully mature females. This shows that vitellogenin (lipoprotein) is synthesized rapidly to its maximum in the hepatopancreas when the ovary is immature, but as maturity commences, the lipids in the hepatopancreas start decreasing, as they are transported through the hemolymph to the developing oocytes. The increase of these contents in the ovaries with maturation shows that the oocytes absorb vitellogenin from the hemolymph, which is converted to vitellin in fully matured oocytes.

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## References

Antunes, G.F., Amaral, A.P.N., Ribarcki, F.P., Wiilland, E.F., Zancan, D.E. and Vinagre, A.S. 2010. Seasonal variations in the biochemical composition and reproductive cycle of the ghost crab *Ocypode quadrata* (Fabricius, 1787) in Southern Brazil. J. Exp. Zool. 313A: 280–291.

Avarre, J.C., Michelis, R., Tietz, A. and Lubzens, E. 2003. Relationship between vitellogenin and vitellin in a marine shrimp (*Penaeus semisulcatus*) and molecular characterization of vitellogenin complementary DNAs. Biol. Reprod. 69: 355–364.

Ayub, Z. and Ahmed, M. 1992. Maturation and spawning of some penaeid shrimps of Pakistan (Arabian Sea). Mar. Res. 1: 29–35.

Ayub, Z. and Ahmed, M. 2002a. A description of the ovarian development stages of penaeid shrimps from the coast of Pakistan. Aqua. Res. 33: 767–776.

Ayub, Z. and Ahmed, M. 2002b. Maturation and spawning of four commercially important penaeid shrimps of Pakistan. Indian J. Mar. Sci. 31: 119–124.

Barnes, H. and Blackstock, J. 1973. Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanillin method for total lipids. J. Exp. Mar. Biol. Ecol. 12: 103–118.

Brown, A. Jr and Patlan, D. 1974. Colour changes in the ovaries of penaeid shrimps as a determinant of their maturity. Mar. Fish. Rev. 36: 23–26.

Brown, M., Sieglaff, D. and Rees, H. 2009. Gonadal ecdysteroidogenesis in Arthropoda: occurrence and regulation. Annu. Rev. Entomol. 54: 105–125.

Castille, F.L. and Lawrence, A.L. 1989. Relationship between maturation and biochemical composition of the gonads and digestive glands of the shrimps *Penaeus aztecus* Ives and *Penaeus setiferus* (L.). J. Crust. Biol. 9: 202–211.

Cavalli R.O., Tamtin, M., Lavens, P. and Sorgeloos, P. 2001. Variations in lipid classes and fatty acid content in tissues of wild *Macrobrachium rosenbergii* (de Man) females during maturation. Aquaculture 193: 311–324.

Chang, E., Chang, S. and Mulder, E. 2001. Hormones in the lives of crustaceans: an overview. American Zool. 41: 1090–1097.

Charniax-Cotton, C.H. 1985. Vitellogenesis and its control in malacostracan crustacea. American Zool. 25: 197–206.

Chen, J.C., Liu, P.C., Lin, Y.S. and Lee, C.K. 1988. Super intensive culture of red-tailed shrimp *Penaeus penicillatus*. J. World Aqua. Soc. 19: 127–131.

Clarke, A. 1977. Seasonal variations in total lipid content of *Chorismus antarcticus* (Crustacea-Decapoda) at South Georgia. J. Exp. Mar. Biol. Ecol. 27(1): 93–106.

Cummings, W.C. 1961. Maturation and spawning of the pink shrimp, *Penaeus duorarum* Burkenroad. Trans. American Fish. Soc. 90: 462–468.

Dall, W., Hill, J., Rothlisberg, P.C. and Shaples, D.J. 1990. The Biology of the Penaeidae. Academic Press, San Diego, CA.

Dubios, M., Gilles, K.A., Hamilton J.K., Rebers, P.A. and Smith F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 305–356.

Garcia, F., Gonzalez-Baro, M. and Pollero, R. 2002. Transfer of lipids between hemolymph and hepatopancreas in the shrimp *Macrobrachium borellii*. Lipids 37: 581–585.

Gehring, W.R. 1974. Maturational changes in the ovarian lipid spectrum of the pink shrimp *Penaeus duorarum* Burkenroad. Comp. Biochem. Physiol. A 49: 511–524.

Giese, A.C. 1966. Lipids in the economy of marine invertebrates. Physiol. Rev. 46: 244–290.

Hasek, B.E. and Felder, D.L. 2005. Biochemical composition of ovary, embryo, and hepatopancreas in the grapsoid crabs *Armases cinereum* and *Sesarma* nr. *reticulatum* (Crustacea, Decapoda). Compar. Biochem. Physiol. B 140: 455–463.

Hoang, T. 2001. The banana prawn--the right species for shrimp farming. World Aqua. Magaz. 32: 40–44.

Karakoltsidis, P.A., Zotos, A. and Constantinides, S.M. 1995. Composition of the commercially important Mediterranean finfish, crustaceans, and mollusks. J. Food Composi. Anal. 8: 258–273.

Khan, S.A. and Natarajan, R. 1980. Biochemical variations during the ovarian cycle of estuarine hermit crab *Clibanarius longitarsus*. In: Progress in Invertebrate Reproduction and Aquaculture (Eds. T. Subramoniam and S. Varadarajan). University of Madras, Madras, pp. 149–161.

Khayat, M., Shenker, O., Funkenstein, B., Tom, M., Lubzens, E. and Tietz, A. 1994. Fat transport in the penaeid shrimp *Penaeus semisulcatus* (De Haan). Israel J. Aqua. 46: 22–32.

Komatsu, M., Ando, S. and Teshima, S.I. 1993. Comparison of hemolymph lipoproteins from four species of Crustacea. J. Exp. Zool. 266: 257–265.

Kulkarni, G.K. and Nagabhushanam, R. 1979. Mobilization of organic reserves during ovarian development in a marine penaeid prawn *Parapenaeopsis hardwickii* (Miers) (Crustacea, Decapoda, Penaeidea). Aquaculture 18: 373–377.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

Mak, A.S.C., Choi, C.L., Tiu, S.H.K., Hui, J.H.L., He, J.G., Tobe, S.S. and Chan, S.M. 2005. Vitellogenesis in the red crab *Charybdis feriantus*: hepatopancreas-specific expression and farnesoic acid stimulation of vitellogenin gene expression. Mol. Reprod. Dev. 70: 288–300.

Marine Fisheries Department. 2006. Handbook of Fisheries Statistics of Pakistan, Vol. 19. Marine Fisheries Department, Government of Pakistan, Karachi, Pakistan.

Mourente, G., Medina, A., Gonzalez, S. and Rodriguez, A. 1994. Changes in lipid class and fatty acid contents in the ovary and midgut gland of the female fiddler crab *Uca tangeri* (Decapoda, Ocypodidae) during maturation. Mar. Biol. 121: 187–197.

Nagabhushanam, R. and Farooqui, U.M. 1982. Mobilization of protein, glycogen and lipid during ovarian maturation in marine crab, *Scylla serrata* Forsskal. Indian J. Mar. Sci. 11: 184–186.

Nagaraju, G.P.C. 2007. Is methyl farnesoate a crustacean hormone? Aquaculture 272: 39–54.

Nagaraju, G.P.C. 2011. Reproductive regulators in decapods crustacean: an overview. J. Exp. Biol. 214: 3–16

Nagaraju, G.P.C., Kumari, N.S., Prasad, G.L.V., Rajitha, B., Meenu, M., Rao, M.S. and Naik, B.R. 2009. Structural prediction and analysis of VIH-related peptides from selected crustacean species. Bioinformation 4: 6–11.

Nisa, K.U. and Sultana, R. 2010. Variation in the proximate composition of shrimp, *Fenneropenaeus penicillatus* at different stages of maturity. Pakistan J. Biochem. Molec. Biol. 43: 135–139.

Oberdorster, E., Rice, C.D. and Irwin, L.K. 2000. Purification of vitellin from grass shrimp *Palaemonetes pugio*, generation of monoclonal antibodies, and validation for the detection of lipovitellin in Crustacea. Comp. Biochem. Physiol. C 127: 199–207.

Okumura, T., Yamano, K. and Sakiyama, K. 2007. Vitellogenin gene expression and hemolymph vitellogenin during vitellogenesis, final maturation, and oviposition in female kuruma prawn, *Marsupenaeus japonicus*. Comp. Biochem. Physiol. A 147: 1028–1037.

Palacios, E., Ibarra, A.M. and Racotta, I.S. 2000. Tissue biochemical composition in relation to multiple spawning in wild and pond-reared *Penaeus vannamei* broodstock. Aquaculture 185: 353–371.

Peixoto, S., Cavalli, R.O., D'Incao, F., Milach A.M. and Wasielesky, W. 2003. Ovarian maturation of wild *Farfantepenaeus paulensis* in relation to histological and visual changes. Aqua. Res. 34: 1255–1260.

Phiriyangkul, P. and Utarabhand, P. 2006. Molecular characterization of a cDNA encoding vitellogenin in the banana shrimp, *Penaeus* (*Litopenaeus*) *merguiensis* and sites of vitellogenin mRNA expression. Molec. Repro. Devel. 73: 410–423.

Pillay, K.K. and Nair, N.B. 1973. Observations on the biochemical changes in gonads and other organs of *Uca annulipes*, *Portunus pelagicus* and *Metapenaeus* affinis (Decapoda: Crustacea) during the reproductive cycle. Mar. Biol. 18: 167–198.

Qingbo, H., Jiwen, Q., Jimin, H. and Degong, Y. 1988. Effect of salinity on the 2-crop culture of penaeid shrimp. Chinese J. Oceano. Limnol. 6: 15–21.

Raviv, S., Parnes, S., Segall, C., Davis, C. and Sagi, A. 2006. Complete sequence of *Litopenaeus vannamei* (Crustacean: Decapoda) vitellogenin cDNA and its expression in endocrinologically induced sub-adult females. Gen. Comp. Endocrinol. 145: 39–50.

Read, G.H.L. and Caulton, M.S. 1980. Changes in mass and chemical composition during the moult cycle and ovarian development in immature and mature *Penaeus indicus* Milne Edwards. Comp. Biochem. Physiol. B 66: 431–437.

Rosa, R. and Nunes, M.L. 2002. Biochemical changes during the reproductive cycle of the deep-sea decapod *Nephrops norvegicus* on the south coast of Portugal. Mar. Biol. 141: 1001–1009.

Rosa, R. and Nunes, M.L. 2003. Biochemical composition of deep-sea decapod crustaceans with two different benthic life strategies of the Portuguese south coast. Deep Sea Res. 50: 119–130.

Sikorski, Z.E., Kolakowska, A. and Burt, J.R. 1990. Post harvest biochemical and microbial changes. In: Seafood: Resources, Nutritional, Composition and Preservation (Ed. Z.E. Sikorski). CRC Press, Boca Raton, FL, pp. 55–75.

Sriket, P., Benjakul, S., Visessanguan, W. and Kijroongrojana, K. 2007. Comparative studies on chemical composition and thermal properties of black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*) meats. Food Chem. 103: 1199–1207.

Suneetha, Y., Reddy, P.S., Jyothi, P.N. and Reddy, M.S. 2009. Proximal changes during reproduction process of the penaeid prawn, *Penaeus monodo*n. World J. Fish Mar. Sci. 1: 333–337.

Tan-Fermin, J.D. and Pudadera, R.A. 1989. Ovarian maturation stages of the wild giant tiger prawn, *Penaeus monodon* Fabricius. Aquaculture 77: 229–242.

Tiu, S.H.K., Hui, J.H.L., Mak., A.S.C., He., J.G. and Chan., S.M. 2006. Equal contribution of hepatopancreas and ovary to the production of vitellogenin (Pm Vg1) transcripts in the tiger shrimp, *Penaeus monodon.* Aquaculture 254: 666–647.

Tuck, I.D., Taylor, A.A., Atkinson, R.J.A., Gramitto, M.E. and Smith C. 1997. Biochemical composition of *Nephrops norvegicus*: changes associated with ovary maturation. Mar. Biol. 129: 505–511.

Van Zalinge, N.P., Khaliluddin, M. and Khan, W. 1987. Description of the shrimp fishery including a stratified sampling scheme for shrimp landing and effort at Karachi Fish Harbour. FI: DP/ PAK/ 77/ 033 Field Document 7. FAO, Rome.

Vazquez-Boucard, C.G., Levy, P., Ceccaldi, H.H. and Brogren, C.H. 2002. Developmental changes in concentration of vitellin, vitellogenin, and lipids in hemolymph, hepatopancreas and ovaries from different ovarian stages of Indian white prawn *Fenneropenaeus indicus*. J. Exp. Mar. Biol. Ecol. 281: 63–75.

Yano, I. 1988. Oocyte development in the kuruma prawn *Penaeus japonicus*. Mar. Biol. 99: 547–553.

Zupanovic, S. 1971. Shrimp Explorations off the Coast of West Pakistan. FAO, Rome.