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BIOCHEMICAL COMPOSITION OF HEPATOPANCREAS AND MUSCLES DURING MOULT STAGES IN THE PENAEID SHRIMPS, *FENNEROPENAEUS MERGUIENSIS* AND *F. PENICILLATUS* (CRUSTACEA: DECAPODA)

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ABSTRACT

The object of the present study was to quantify the concentration of protein, carbohydrate and lipid in the hepatopancreas and muscle of *Fenneropenaeus merguiensis* and *F. penicillatus* during the moulting stages, post moult (stages A and B), intermoult (stage C) and premoult (stage D). The protein concentrations in the hepatopancreas showed significant differences between males and females of *F. merguiensis*. However, the lipid and carbohydrate were not significantly different between the two sexes in this species. The protein, carbohydrate and lipid concentrations in the hepatopancreas of *F. penicillatus* were significantly different in two sexes. The protein and carbohydrate concentrations in the muscles of males and females *F. penicillatus* were significantly different but in *F. merguiensis* these concentrations were not significantly different. The lipid concentrations were different between two sexes in both species. The protein and carbohydrate concentrations in hepatopancreas of *F. merguiensis* and *F. penicillatus* were either of same order or slightly different during stages B and C, but significantly higher during molting stage D. The lipid concentrations in the hepatopancreas of these two species varied during the three molting stages, being lowest during stage B and highest during stage D. In two species, the protein, carbohydrate and lipid concentrations in the muscles were almost similar during moult stages B and C but higher in stage D.

Key words: Penaeid shrimp, moult stages, proximate composition, hepatopancreas, muscle

INTRODUCTION

The physiology and reproduction in crustaceans is directly linked to the moulting cycle. The accumulation and mobilization of organic reserves, lipid, protein and carbohydrates are important constituents required during the moulting cycle in crustaceans. The reserves are not only needed for the formation of new skeleton but also provide the energy needed during the process of moulting. A proper utilization of these organic reserves makes certain that the moult cycle is completed successfully and there is good growth at each moult (Richard, 1980; Barclay *et al*., 1983).

In order to understand the biochemical and biological changes, it is important to identify the stages of the moult cycle (Smith and Dall, 1985). The moulting in crustaceans was divided into five main stages, that is, immediate postmoult (stage A), postmoult (stage B), intermoult (stage C), premoult (stage D) and ecdysis (stage E) (Drach, 1939). Two types of intermoult stages usually occurred in crustaceans (Knowles and Carlisle, 1956), anecdysis, where there is a long intermoult period and diecdysis, where there is no clear intermoult period. The penaeid shrimps usually have diecdysal moulting cycle (Smith and Dall, 1985). Among the penaeids, criteria for assessing moult stages are described for *Penaeus duorarum* (Schafer, 1968), *P. merguiensis* (Longmuir, 1983), *P. stylirostris* (Huner and Colvin, 1979; Robertson *et al*., 1987), *P. esculentus* (Smith and Dall, 1985) *P. setiferus* (Robertson *et al*., 1987).

Some studies have dealt with the changes in protein, lipid, and carbohydrate content of the midgut gland during the course of the moult cycle in common shore crab *Carcinus meanus* (Heath and Barnes, 1970) and *Astacus zeptodactylus* (Durliat and Vranckx, 1982). According to Passano (1960) the lipids are stored in the digestive gland during the early premoult and are utilized during the late premoult and ecdysis in crustaceans. Similarly an increase in the lipid content of the midgut gland has been reported in premoult crayfish and crabs (O'Connor and Gilbert, 1969) as well as in shrimps (Ando *et al*., 1977). During premoult stage, levels of glucose (Telford, 1968) and lipid (Spindler-Barth, 1976) in the hemolymph of crabs increased markedly as compared to postmoult stage. The decrease of glucose in the postmoult has been related to its utilization as a precursor in chitin synthesis (Meenakshi and Scheer, 1961; Hornung and Stevenson, 1971).

Bursey and Lane (1971) reported in *P. duorarum* that the protein contents of the hemolymph was the lowest during the postmoult stages A and B and increased to maximum during premoult stage D and then declined again before moult. Read and Caulton (1980) while studying the changes in chemical composition during moult cycle in *P. indicus* found that protein and lipid contents remained almost similar during postmoult stages A and B but started increasing significantly to reach maximum at premoult stage D.

Diwan and Usha (1985) reported that during the moulting cycle of *P. japonicus* the lipid concentration in hepatopancreas reached a maximum at beginning of premoult period and decreased after that. Diwan and Usha (1987) studied the quantitative variations in the lipid, cholesterol, protein and glycogen contents in the muscle, hepatopancreas and hemolymph of *Penaeus indicus* and reported that the lipid and protein contents of hepatopancreas and muscles increased from stage A and reached to maximum at stage D1 and then decreased during the late premoult stage D4. The glycogen contents were also found to be highest in early premoult, stage D1 with reduction at stage D4 which showed that it has been mobilized for growth of post exuvial layers. In the present study quantitative variations in the protein, lipid and carbohydrate in two species of shrimp, *Fenneropenaeus merguiensis* and *F. penicillatus* during different stages of the moult cycle were estimated.

MATERIAL AND METHODS

Adult females and males of *F. merguiensis* and *F. penicillatus* were procured from the fishermen operating their trawler in the vicinity of Karachi, Pakistan. The shrimps were brought to the laboratory in ice-box. In the laboratory the shrimps were measured for their carapace length (cm) and weight (gm).

Biochemical analysis of muscles and hepatopancreas during moulting stages

The females of *F. merguiensis* and *F. penicillatus* with immature ovaries were selected to estimate the biochemical changes during moulting stages, as it has been reported that the concentrations of protein, lipid and carbohydrate of the hepatopancreas changes in relation to ovarian maturation stages in various crustacean species (Tehsima and Kanazawa, 1983; Galois, 1984; Tuck *et al*., 1997; Wen *et al*., 2001). The size range of animals that were used to identify the moulting stage in *F. merguiensis* was 35 to 65 mm carapace length (CL) and 17.4 to 50.0 g total weight (TW) for females and 35 to 55 mm CL and 9.3 to 40.0 g TW in males. The size range of *F. penicillatus* females was 40 to 67 mm CL and 27.4 to 50.0 g TW and of males was 40 to 57 mm CL and 8.9 to 37.0 g TW.

The identification of moulting stages in the crustacean is based on the observations of the degree of hardness of the exoskeleton and microscopic examination of the transparent edge of the inner uropod in the region adjacent to the telson tip where epidermal withdrawal and development of new setae can be observed (Drach, 1939; Passano, 1960; Drach and Tchernigovtzeff, 1967; Yamaoka and Scheer, 1970; Lyle and MacDonald, 1983). Similarly shrimps can be staged for moulting by examining the pleopods (Longmuir, 1983) or the uropods (Smith and Dall, 1985).

In the present study for moulting stages the edge of the inner uropod in the region adjacent to the telson tip was examined (Fig. **1A**) using a Leica microscope with 40x magnification. The following criteria was used to identify the moulting stages in *F. merguiensis* and *F. penicillatus* (Smith and Dall, 1985).

Stage A (immediate postmoult)

Cuticle slippery, still soft and membranous. Cellular matrix fills setae and setal bases. In later stage the cellular matrix begins to retract from proximal end of setae.

Stage B (Postmoult)

The exoskeleton is relatively hard though flexible. Constrictions in setal lumen begin and setal cone or plug are not yet clearly developed or visible (Fig. **1B**).

Stage C (intermoult)

Exoskeleton becomes very rigid. Setal cones present and clearly visible in most setae. Epidermis fills the bases of the setae and has a translucent border that extends round the nodes as well as the bases (Fig. **1C**).

Stage D (Premoult)

It is determined by the withdrawal of epidermis from setal bases and a straight epidermal line is observed below setal bases. In some cases a translucent zone between the epidermis and the setal bases is formed and can be observed (Fig. **1D**).

For the biochemical estimation, the wet weight of shrimp muscle and their respective hepatopancreas were washed in phosphate buffer (pH 7) and then the homogenate was prepared in the same buffer to estimate the concentrations of protein, carbohydrate and lipid. Triplicate biochemical analyses were conducted using the standard procedures. Protein was estimated by colorimetric method as described in Lowry *et al*. (1951). Carbohydrate was estimated by the Phenol-Sulphuric acid method of Dubois *et al*. (1956). Total lipids were estimated by Sulphovanillin method of Barnes and Blackstock (1973).

Statistical Analysis

Two-factor ANOVA was used in *F. merguiensis* and *F. penicillatus* to test for differences in biochemical composition of hepatopancreas and muscle with sex and moulting stages as factors. If factors were significant ($P < 0.05$), they were tested with Tukey test (multiple comparison test).

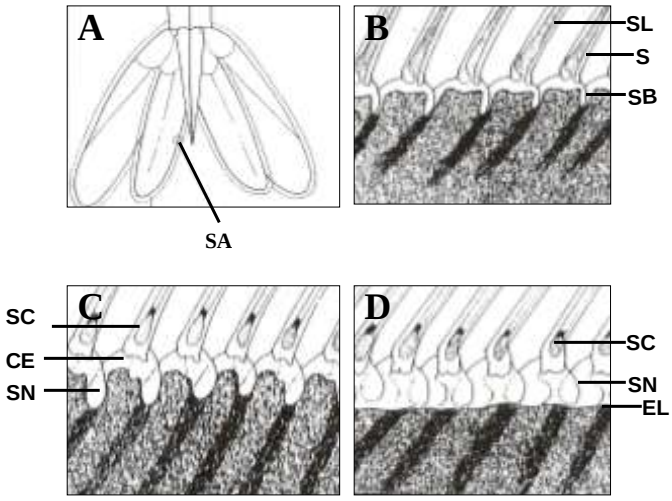

Fig. 1. A- Uropod showing the sample area (SA) used for moult staging. The line drawings of B, C and D stages. SA, sample area; SL, setal lumen; S. setal shaft; SB, setal base; SC, setal cone; SN, setal node; EL, epidermal line; CE, clear cutical edge of uropod (Smith and Dall, 1985)

RESULTS

Biochemical composition of hepatopancreas during moulting stages

The average concentrations of protein, carbohydrate and lipid in the hepatopancreas of *F. merguiensis* and *F. penicillatus* during the moulting stages are shown in Table 1. The concentrations of these component for the pooled data showed that in *F. merguiensis* females the concentration of protein in the hepatopancreas varied from 70 to 117.5 mg l^{-1}, that of carbohydrate from 5.3 to 21 mg l^{-1} and lipid from 85 to 200 mg l^{-1}. In *F. merguiensis* males the concentrations of protein, carbohydrate and lipid in the hepatopancreas varied from 72 to 110 mg l^{-1}, 7 to 20 mg $l^{-1}$and 90 to 200 mg l^{-1} (9.0-20.0%), respectively. In females of *F. penicillatus* the concentrations of protein in the hepatopancreas varied from 72 to 120 mg l^{-1}, that of carbohydrate from 8 to 22 mg l^{-1} and lipid from 90-190 mg l^{-1}. In males of *F. penicillatus* the concentrations of protein, carbohydrate and lipid in the hepatopancreas varied from 80 to 110 mg l^{-1}, 9 to 18 mg l^{-1} and 85 to 215 mg l^{-1}, respectively.

The protein concentrations in the hepatopancreas showed significant differences between males and females of *F. merguiensis*. However, the lipid and carbohydrate concentrations were not significantly different between the two sexes in this species (Table 2). The protein and carbohydrate concentrations in the hepatopancreas of *F. merguiensis* males and females was highest during moulting stage D, while lipid concentrations increased significantly in hepatopancreas as moulting stages advanced in *F. merguiensis* males and females.

The protein, carbohydrate and lipid concentrations in the hepatopancreas showed significant differences between males and females of *F. penicillatus* (Table 3), The protein concentrations in the hepatopancreas of *F. penicillatus* males and females during three moulting stages were almost similar. During the moulting cycle in males and females of *F. penicillatus*, the carbohydrate concentrations was highest in stage D, whereas lipid concentration varied significantly as moulting stages advanced in both sexes of *F. penicillatus*.

Biochemical composition of muscles during moulting stages

The average concentrations of protein, carbohydrate and lipid in the hepatopancreas of *F. merguiensis* and *F. penicillatus* during the moulting stages are shown in Table 4. In the present study the pooled data in the muscle of *F. merguiensis* females showed that the concentrations of protein, carbohydrate and lipid varied from 60 to 187 mg l^{-1}, 2.5 to 12 mg l^{-1} and 2.5 to 12 mg l^{-1}, respectively. The concentrations of protein in the muscle of *F. merguiensis* males varied from 60 to 200 mg l^{-1}, that of carbohydrate from 3.5 to 13 mg l^{-1} and of lipid from 6.0 to 9.5 mg l^{-1}. In

females of *F. penicillatus* the concentrations of protein in the muscle varied from 85 to 202 mg l^{-1}, that of carbohydrate from 4 to 12 mg l^{-1} and lipid from 5 to 12 mg l^{-1}. In the males of *F. penicillatus* the concentrations of protein, carbohydrate and lipid in the muscles varied from 57.5 to 180 mg l^{-1}, 3 to 12 mg l^{-1} and 6.0 to 10 mg l^{-1}, respectively.

Table 1. The average concentrations of protein, carbohydrate and lipid (with standard deviation) in the hepatopancreas during the moult stages in females and males of *F. merguiensis* and *F. penicillatus*.

	Females			Males		
	Moulting stages					
F. merguiensis	Stage B	Stage C	Stage D	Stage B	Stage C	Stage D
Protein	75.5 ± 5.0 a	81.6 ± 8.6 ab	89.6 ± 14.3 b	107.4 ± 30.9 a	78.4 ± 17.8 a	100.4 ± 14.6 a
Carbohydrate	10.6 ±6.6 a	10.1 ± 5.6 ab	15.1 ± 5.4 ab	16.6 ± 3.8 ab	14.1 ± 1.9 a	15.9 ± 2.0 b
Lipid	96.5 ±5.8 a	121.3 ± 17.6 b	173.3 ± 15.9 b	128.6 ± 11.3 a	158.5 ± 6.5 a	183.7 ± 15.0 c
F. penicillatus	Stage B	Stage C	Stage D	Stage B	Stage C	Stage D
Protein	80.7 ±10.2 a	92.6 ± 13.4 a	95.1 ±14.9 a	95.8 ± 6.8 a	98.2 ± 5.2 a	107.5 ± 2.1 a
Carbohydrate	13.4 ± 1.3 a	17.6 ± 2.5 a	17.8 ± 2.9 a	15.3 ± 1.3 ab	14.2 ± 1.5 a	16.3 ± 1.3 b
Lipid	111.6 ± 9.3 a	136.3 ± 13.0 a	170.9 ± 8.0 a	132.5 ± 11.9 a	160.0 ± 3.5 a	206.3 ± 25.6 c

Figures followed by the same letters are not significantly different at $p<0.05$ as given by the Turkey test.

Table 2. Two way analysis of variance to test differences in biochemical concentration of the hepatopancreas of males and females *F. merguiensis* during moult stages.

Biochemical composition	Source of Variation	df	F ratio	p value
Protein	Sex	1	24.747	0.000
	Moult stage	2	8.435	0.001
	Sex * moult stage	2	0.830	NS
Carbohydrate	Sex	1	2.820	NS
	Moult stage	2	11.626	0.000
	Sex * moult stage	2	3.485	0.038
Lipid	Sex	1	3.065	NS
	Moult stage	2	111.742	0.000
	Sex * moult stage	2	0.126	NS

Significant at $P< 0.05$ level; NS = not significant.

Table 3. Two way analysis of variance to test differences in biochemical concentration of the hepatopancreas of males and females *F. penicillatus* during moult stages.

Biochemical composition	Source of Variation	df	F ratio	p value
Protein	Sex	1	25.255	0.000
	Moult stage	2	6.706	0.003
	Sex * moult stage	2	0.405	NS
Carbohydrate	Sex	1	8.494	0.005
	Moult stage	2	28.690	0.000
	Sex * moult stage	2	10.505	0.000
Lipid	Sex	1	8.809	0.004
	Moult stage	2	145.246	0.000
	Sex * moult stage	2	1.769	NS

Significant at $P< 0.05$ level; NS = not significant.

Table 4. The average concentrations of protein, carbohydrate and lipid (with standard deviation) in the muscles during the moult stages in females and males of *F. merguiensis.* and *F. penicillatus.*

	Females			Males		
	Moulting stages					
F. merguiensis	Stage B	Stage C	Stage D	Stage B	Stage C	Stage D
Protein	80.5 ± 16.5 a	90.5 ± 22.5 a	139.5 ± 42.4 b	99.7 ± 35.2 a	103.7 ± 36.2 a	111.2 ± 42.2 a
Carbohydrate	4.4 ± 3.0 a	4.8 ± 3.0 a	9.0 ± 3.7 b	6.5 ± 2.7 a	6.3 ± 1.9 a	7.7 ± 2.8 a
Lipid	7.6 ± 2.6 a	8.2 ± 2.3 a	9.4 ± 2.6 b	5.0 ± 1.0 a	7.6 ± 0.6 a	9.3 ± 1.0 b
F. penicillatus	Stage B	Stage C	Stage D	Stage B	Stage C	Stage D
Protein	108.0 ± 11.7 a	130.0 ± 18.5 b	144.5 ± 30.3 b	96.6 ± 14.2 a	90.9 ± 6.9 a	91.8 ± 23.4 a
Carbohydrate	8.1 ± 1.0 a	8.9 ± 1.5 a	9.5 ± 1.6 a	4.9 ± 1.4 a	6.8 ± 3.3 a	7.2 ± 0.9 a
Lipid	9.1 ± 0.8 a	9.8 ± 0.8 a	10.5 ± 1.1 b	5.2 ± 0.8 a	7.4 ± 1.4 a	10.3 ± 0.6 b

Figures followed by the same letters are not significantly different at $p<0.05$ as given by the Turkey test.

Table 5. Two way analysis of variance to test differences in biochemical concentration in the muscles of males and females *F. merguiensis* during moult stages.

Biochemical composition	Source of Variation	df	F ratio	p value
Protein	Sex	1	0.064	NS
	Moult stage	2	12.175	0.000
	Sex * moult stage	2	2.744	NS
Carbohydrate	Sex	1	0.124	NS
	Moult stage	2	9.244	0.000
	Sex * moult stage	2	1.695	NS
Lipid	Sex	1	12.255	0.001
	Moult stage	2	12.203	0.000
	Sex * moult stage	2	0.085	NS

Significant at $P< 0.05$ level; NS = not significant.

Table 6. Two way analysis of variance to test differences in biochemical concentration of the muscles of males and females *F. penicillatus* during moult stages.

Biochemical composition	Source of Variation	df	F ratio	p value
Protein	Sex	1	6.653	0.013
	Moult stage	2	9.379	0.000
	Sex * moult stage	2	1.002	NS
Carbohydrate	Sex	1	7.510	0.008
	Moult stage	2	3.364	0.042
	Sex * moult stage	2	0.343	NS
Lipid	Sex	1	86.168	0.000
	Moult stage	2	20.470	0.000
	Sex * moult stage	2	0.649	NS

Significant at $P< 0.05$ level; NS = not significant.

No significant difference in protein and carbohydrate concentrations of the muscle was found between males and females of *F. merguiensis* (Table 5.), while the lipid concentration was different between two sex of this species. The protein and carbohydrate concentrations in the muscles of *F. merguiensis* males did not show significant differences during the moulting stages, while the lipid concentration in the muscles of this species was found to be highest in moulting stages D, while during stages B and C the lipid concentration was similar. The protein, lipid and

carbohydrate concentrations in the muscles of *F. merguiensis* females were highest during moulting stage D, while during stages B and C, these constituents were almost similar.

The protein, carbohydrate and lipid concentrations in the muscle showed significant differences between males and females of *F. penicillatus* (Table 6). In the muscles of *F. penicillatus* males, the protein and carbohydrate concentrations did not vary significantly as moulting stages advanced, while the lipid concentrations in muscles was similar during stages B and C and varied significantly and was higher during stage D. In the muscles of *F. penicillatus* females, the protein concentration was lowest during stage B and almost similar during stages C and D. The carbohydrate and lipid concentrations, in the muscles of *F. penicillatus* females were highest during moulting stage D and similar during stages B and C.

DISCUSSION

In the present study no shrimp of *F. merguiensis* and *F. penicillatus* was found in the moulting stage A. This stage begins when the animal shed its skeleton and the cuticle is very soft and slippery. As the animal is vulnerable to predator, therefore, there is a possibility that the animals remain buried and thus no shrimp in moult stage A was caught.

In the present study the protein and carbohydrate concentrations in hepatopancreas of *F. merguiensis* and *F. penicillatus* were either same or slightly different during stages B and C, but significantly higher during molting stage D. The lipid concentrations in the hepatopancreas of these two species varied during the three molting stages, being lowest during stage B and highest during stage D. Therefore, the highest concentrations of protein, carbohydrate and lipid in the hepatopancreas of *F. merguiensis* and *F. penicillatus* were observed during the molting stage D in the present study which is similar to number of other studies in which the highest concentrations of these constituents were found in the hepatopancreas during moulting stage D. Total protein content in the hepatopancreas of crayfish *Astacus leptodactylus* was low in postmolt stages A and B, increased in stage C, decreased again from D0 to D1 and then increased in D2 to D3 (Durliat and Vranckx, 1982). Similarly Diwan and Usha (1985) found that the lipid concentration in hepatopancreas of *P. japonicus* reached a maximum at beginning of premoult period and after that it decreased. Diwan and Usha (1987) reported that the lipid and protein contents of hepatopancreas in *Penaeus indicus* increased from stage A and reached to maximum at stage D1 and then decreased during the late premoult stage D4. The glycogen contents in *P. indicus* were highest in early premoult, stage D1 which later reduced at stage D4, which showed that it has been mobilized for growth of post exuvial layers (Diwan and Usha, 1987).

The protein, carbohydrate and lipid concentrations in muscles of *F. merguiensis* and *F. penicillatus* were either same or slightly different during moulting stages B, C and D. On the whole during the moulting stage D, the shrimps showed the highest concentrations of protein, carbohydrate and lipid concentrations in the muscles, which is similar to the study reported by Read and Caulton (1980) that in tissue of *P. indicus*, protein and lipid contents remained almost similar during postmosult (stages A & B) but started increasing significantly to reach maximum at premoult (stage D). Diwan and Usha (1987) reported that the lipid, protein and glycogen contents in the muscle of *P. indicus*, reached to maximum at early postmoult stage D1 and then decreased during the late premoult stage D4.

REFERENCES

Ando, T., A. Kanazawa, S. Teshima, J. Patrois and H.J. Ceccaldi (1977). Variation in the lipids of tissues during the molting cycle of prawn. *Bull. Jap. Soc. Scient. Fish*., 43:1445-1449.

Barclay, M.C., W. Dall and D.M. Smith (1983). Changes in lipid and protein during starvation and the moulting cycle in the tiger prawn, *Penaeus esculatus* (Haswell). *J. Exp. Mar. Biol. Ecol*., 69: 229-244.

Barnes, H. and J. Blackstock (1973). Estimation of lipid in marine animals and tissues: Detailed investigation on the sulpho-phospho vanillin method for total lipid. *J. Exp. Mar. Biol. Ecol*., 2: 103-118.

Bursey, C.R. and C.E. Lane (1971). Ionic and protein Concentration changes during the moult cycle of *Penaeus duorarum. Comp. Biochem. Physiol*., 40: 155-162.

Diwan, A.D. and T. Usha (1985). Characterization of moult stages of *Penaeus indicus* based on developing uropod setal and some closely allied structures. *Indian J. Fish* ., 32: 275-279.

Diwan, A.D. and T. Usha (1987). Mobilization of Organic Reserves during Moult Cycle in the Prawn *Penaeus indicus* (H.Milne Edwards). *Indian J. Mar. Sci*., 16: 65-68.

Drach, P. (1939). Mue et cycle d' intermeue chez les crustace's decapods. *Ann. Inst. Oceanogr. Paris N. S*., 19: 103-391.

Drach, P. and C. Tchernigovtzeff (1967). Sur la methode de determination des stades d'intermue et son application general aux Crustaces Vie Milieu Ser. *A Biol. Mar*., 18: 595-610.

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

Durliat, M. and R. Vranckx (1982). Proteins of aqueous extracts from the hepatopancreas of *Astacus leptodactylus*. I. changes in proteins during the molt cycle. *Comp. Biochem. Physiol*., 71: 155-163.

Galois, R.G. (1984). Variations de la composition lipidique tissulaire au cours de la vitellogenese chez la crevette *Penaeus indicus* Milne Edwards. *J. Exp. Mar. Biol. Ecol*., 84: 155-166.

Heath, J.R. and H. Barnes (1970). Some changes in biochemical composition with season and during molt cycle of the common shore crab, *Carcinus maenas* (L.). *J. Exp. Mar. Biol. Ecol*., 5: 199-233.

Hornung, D. and J. Stevenson (1971). Changes in the rate of chitin synthesis during the crayfish molting cycle. *Comp. Biochem. Physiol*., 40: 341-346.

Huner, J.V. and L.B. Colvin (1979). Observation on the moult cycles of two species of juvenile shrimp, *Penaeus californiensis* and *Penaeus stylirostris* (Decapoda: Crustacea). *Proc. Nat. Shellfish AS. Soc*., 69: 77- 84

Knowles, F.G.W. and D.B. Carlisle (1956). Endocrine control in the crustacean. *Biol. Rev*., 31: 396-473.

Longmuir, E. (1983). Setal development, moult staging and ecdysis in the banana prawn *Penaeus merguiensis. Mar. Biol*., 77: 183-190.

Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall (1951). Protein measurement with the Folin Phenol reagent. *J. Biol. Chem.*, 193: 265-275.

Lyle, W. G., and C. D. MacDonald. 1983. Molt stage determination in the Hawaiian spiny lobster *Panulirus marginatus*. *J. Crusta. Biol*., 3: 208-216.

Meenakshi, V. and B. Scheer (1961). Metabolisum of glucose in the crab *Cancer magister* and *Hemigrapsus nudus. Comp. Biochem. Physiol*., 3: 30-41.

O'Connor J.D. and L.I. Gilbert. (1969). Alteration in lipid metabolism associated with premolt events in a land crab and cray-fish. *Comp. Biochem. Physiol*., 29: 889-904.

Passano, L.M. (1960). Molting and its control. In: *Physiology of Crustacea. I. Metabolism and growth* (Waterman, T. Editor). Academic Press, New York. Pp. 475-536.

Read, G.H.L. and M.S. Caulton (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.

Richard, P. (1980). Le metabolisme amino-acids de Palaemon serratus: Variatins des acides amines libres du muscle et de I hepatopancreas au Cours du cycle de mue. *Comp. Biochem. Physiol*., 67: 553-560

Robertson, I., W. Bray, J. Leung-Trujillo and A.L. Lawrence (1987). Practical molt staging of *Peneus setiferus* and *Penaeus stylirostris*. *J. World Aqua. Soc*., 18: 180-185.

Schafer H.J. (1968). The determination of some stages of the molting cycle of *Penaeus duorarum*, by microscopic examination of the setae of the endopeptides of pleopods. *FAO Fish. Rep*., 57: 381-391.

Smith, D.M., and W. Dall (1985). Moulting staging the tiger prawn *Penaeus esculatus.* In *Second Australian National Prawn Seminar*. P. C. Rothlisberg, B.J. Hill, and D.J. Staple, eds.. NPS2, Cleveland, Queensland, Austrialia. Pp. 85-93.

Spindler-Barth, M. (1976). Changes in the chemical composition of the common shore crab, *Carcinus maenas. J. Comp. Physiol*., 105: 197-205.

Telford, M. (1968). The identification of sugars in the blood of three species of Atlantic crabs. *Biol. Bull*., 135: 674-584.

Teshima, S. and A. Kanazawa (1983). Variation in lipid composition during the ovarian maturation of the prawn. *Bull. Jap. Soc. Sci. Fish*., 49:957-962.

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

Wen, X. B., L.Q. Chen, C.X. Ai, Z.L. Zhou and H.B. Jiang (2001). Variation in lipid composition of Chinese mitten-handed crab, *Eriocheir sinensis* during ovarian maturation. *Comp. Biochem. Physiol.*, B 130: 95-104.

Yamaoka, L.H. and B.T. Scheer (1970). Chemistry of growth and development in crustaceans. In: *Chemical Zoology.* Academic Press, New York. Pp. 321-341.

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