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Variation in the Proximate Composition of Shrimp, *Fenneropenaeus penicillatus* at Different Stages of Maturity

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Abstract: *Fenneropenaeus penicillatus* (also called *Penaeus penicillatus)* is an abundant species on Pakistan Coast, locally called as "Jaira". The proximate chemical composition was determined in mid gut gland, ovary and muscle during different stages of ovarian development that was found to be varied during maturation among the different tissues examined. Accumulation of different organic constituents was significant in midgut gland and ovary, whereas, muscle did not show any significant variation during maturation process. The lipid content was highest in the midgut gland as compare to ovary and muscle and the quantity was found increasing throughout the ovarian development in midgut gland and ovary while fluctuating in muscle. The increase in the protein content was not as significant in the midgut gland and muscle as that of the ovary during different stages of sexual maturation. The carbohydrate content did not show any significant change in any tissue though found to be highest in midgut gland as compared to ovary and muscle. The present study suggests that there was no mobilization of lipids from midgut gland to ovaries during ovarian maturation and the lipid increase was due to the intake through diet.

Key words: *Fenneropenaeus penicillatus* · Variation · Proximate chemical composition · Maturity stages

INTRODUCTION

From Pakistan, twenty five species of penaeid shrimps have been recorded [1]. Out of these 25 species, only 12 species are exploited commercially [2]. The annual shrimp catch during 1971-2007 ranged between 16,050 to 34,920 metric tons with an average production of 24,937±4341 metric tons (Fishery Statistics of Pakistan, 2002, unpublished data). *Fenneropenaeus penicillatus* (also called *Penaeus penicillatus)* is a commercially important and abundant species of Pakistan Coast; it constitutes the bulk of the catch of large size category of shrimp (Jaira) together with *F. merguiensis* (also called *P. merguiensis*). The relative abundance and economic importance of *F. penicillatus* in Pakistan and its significance as a suitable candidate species for culture due to its high temperature and salinity tolerance [3] deserves in depth studies on its reproduction and maturation. In Pakistan, work on the species remained restricted mostly to the taxonomic [4,5] and biological studies [6-10]. Data related to biochemical studies are scarce and refer only to the determination of proximate biochemical composition of the tail muscle of *Penaeus* spp [11-14]. Such data have limited significance, since it is not species specific or related to either physiological (e.g the stage of gonadal maturation or moulting cycle) or ecological parameters (e.g time of the year, temperature and food availability) [15].

The changes in proximate composition of shrimp ovary, midgut gland and muscles (gonads) have been regarded as an indicator of progress of maturation process due to the accumulation of organic constituents. Such changes have been previously observed in shrimps, *Metapenaeus affinis*, *Penaeus indicus*, *Parapenaeopsis hardwicki*, *P. aztecus* and *P. setiferus* [16-19]. The variations in the biochemical composition have been documented as the species specific; in *P. aztecus* protein content and carbohydrate content of the midgut gland increased with no change in lipid content during maturation, whereas in *P. setiferus,* decrease in the lipid content in midgut gland was associated with maturation [19].

The proximate composition of the shrimps, crustaceans and other aquatic organisms has found to be

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varied due to the seasonal factors, climatic factors, geographic factors, habitat, developmental stage, sex, sexual maturation [16,20-26]. The biochemical composition also varied in shell and flesh of *Fenneropenaeus indicus* [27]. Recently Suryavanshi *et al.,* [28] found the decrease in nutritional value of shrimp, *Metapenaeus monoceros* due to the effect of organochlorine pesticide, endosulfan;. the sublethal doses of endosulfan significantly ($P<0.05$) altered the levels of total protein, total carbohydrates, glycogen, total free sugars and total lipids in test shrimps. Concentrations of biochemical components significantly varied with the duration of exposure but were dose-independent ($P<0.05$). In some species of shrimps and other decapods ovarian lipids may be derived from the diet whereas, in some others lipids may be accumulated and later transported to the ovaries during gonad maturation [29]. In view of many variables exerting their affect on the proximate composition, it became quite imperative to study the proximate composition of *F. penicillatus* at different maturity stages, which has not been under taken previously.

In female shrimps maturity stages of the ovary are determined by one or a combination of following factors: (i) visual changes, (ii) histological changes, (iii) gonadal indexes and (iv) biochemical changes. The visual changes were associated with changes in size and color of the ovary and correlated with the histological changes in the ovaries [30-39]. Sultana [39] described the structures of ovary and maturation stages in *P. penicill*atus; five stages have been recognized including the spent on the basis of colour and histological studies. Later Ayub and Ahmed [40-41] recognized six stages of ovarian development on the basis of color and histological observations and include two additional stages, the resorbing and resorbing / developing as compatible to spent stage. For the present study to determine the variation in the proximate composition during maturation, four stages viz, (i) immature (ii) early maturing, (iii) late maturing and iv) ripe were considered leaving the spent or resorbing stages.

MATERIALS AND METHODS

P. penicillatus were collected from commercial catches at Korangi Creek Harbour. Females were sorted according to the maturity stages of the ovary. Shrimps were transported to the laboratory in ice in insulated boxes. Maturity stages of ovary were recognized by visual observation, which is based on color and size of ovary. Following maturity stages were recognized:

Stage 1: Immature (the transparent to opaque un-pigmented)

Stage 2: Early maturing (light green ovaries, the middle lobe not visible)

Stage 3: Late maturing (bright green, swollen and slightly turgid lobes)

Stage 4: Fully mature (dark olive green ovaries, occupies more than 2/3 part dorsally)

The tissues from several individuals were pooled to obtain a sufficient amount for analyses. All samples were stored at - 40°C until analyzed. Protein, moisture and ash were determined as per AOAC method [42]. Carbohydrate was determined by the method by Dubois *et al.,* [43]. Lipid was determined by Folch *et al.,* [44]. The energy content was calculated as: proteins 4.27 kcal / g wet weight, lipids 9.02 kcal /g wet weight, carbohydrates 4.11 kcal / g wet weight (1 kcal = 4.184 kJ) [45].

RESULTS AND DISCUSSION

The variation in the proximate chemical composition of the mid gut gland, ovary and muscle at different maturity stages of *F. penicillatus* have been determined on wet weight basis (Table 1) to elucidate the relationship between biochemical composition and the ovarian maturation. The dry matter in midgut gland increased from 25.4 % (stage I) to 29.65 % (stage IV) of the midgut gland wet weight. Lipid increased progressively from initial level of 4.9 % at stage I to 8.0 % at stage IV, lipid content was found to be highest in the midgut gland as compared to ovary and muscle, whereas protein increased slightly from 17.2 % (stage I) to 18.0 % (stage IV) showing no significant variation throughout the maturation process. Carbohydrate content ranged from 1.9 % (stage I) to 2.1 % (stage II andIII). The carbohydrate was higher in midgut gland than ovary and muscle where it slightly decreased between immature and early mature stages and increase between early mature and fully mature stages of ovarian development. Ash ranged between 1.40 % (stage I) to 1.68 % (stage III). The moisture content decreased from 74.6 % (stage I) to 70.35 % (stage IV) (Figure 1a).). The energy content increased from initial value of 523 kJ (stage I) to 656 kJ (stage IV).Higher concentration of energy was found in midgut gland as compared to other tissues examined. The midgut gland is the main storage organ of organic and inorganic reserves in decapods

Table 1: Variation in the proximate composition (% wet weight) in midgut gland, ovary and muscle of *Fenneropenaeus. penicillatus* during different maturity stages

	Midgut Gland					
Maturity stages	Lipid	Protein	Carbohydrate	Ash	Dry matter	Moisture
Stage I	4.9^{a}	17.2^{a}	1.90^{a}	1.40^{a}	25.40^{a}	74.6^{c}
Stage II	6.5^{b}	17.5^{a}	2.10^{a}	1.47^{a}	27.57^{ab}	72.43^{b}
Stage III	7.5^{c}	17.8^{ab}	2.10^{a}	1.68^{b}	29.08^{b}	70.92^{a}
Stage IV	8.0^{d}	18.0^{b}	2.00^{a}	1.65^{b}	29.65^{b}	70.35^{a}
Percent increase/decrease	63 .0	4.65	5.26	17.85	16.73	-5.69
	Ovary					
Maturity stages	Lipid	Protein	Carbohydrate	Ash	Dry matter	Moisture
Stage I	2.60^{a}	15.9^{a}	0.70^{a}	1.80^{a}	21.00^{a}	79.00^{c}
Stage II	4.30^{b}	17.50^{b}	1.55^{b}	1.90^{a}	25.00^{b}	75.00^{b}
Stage III	6.13^{c}	18.00^{c}	1.17^{ab}	2.20^{b}	27.80	72.20ab
Stage IV	6.80^{c}	18.40^{c}	1.00^{a}	2.00b	28.20	71.80^{a}
Percent increase/decrease	161.5	15.72	42.85	11.11	34.29	-9.11
	Muscle					
Maturity stages	Lipid	Protein	Carbohydrate	Ash	Dry matter	Moisture
Stage I	1.30^{a}	18.40^{a}	0.99^{b}	1.86^{a}	22.53^{a}	77.47^{b}
Stage II	1.50^{b}	19.00^{b}	0.66^{a}	1.85^{a}	22.94^{ab}	77.06^{ab}
Stage III	1.20^{a}	18.68^{a}	0.87^{b}	1.81^{a}	23.20^{b}	76.80^{a}
Stage IV	1.50^{b}	19.00^{b}	0.66^{a}	1.86^{a}	23.00^{b}	77.00^{a}
Percent increase/decrease	15.38	3.26	-33.33	0.0 %	2.08	-0.0606

Data represent mean values. Values within a column with same superscript letters are not significantly different. ($P<0.05$)

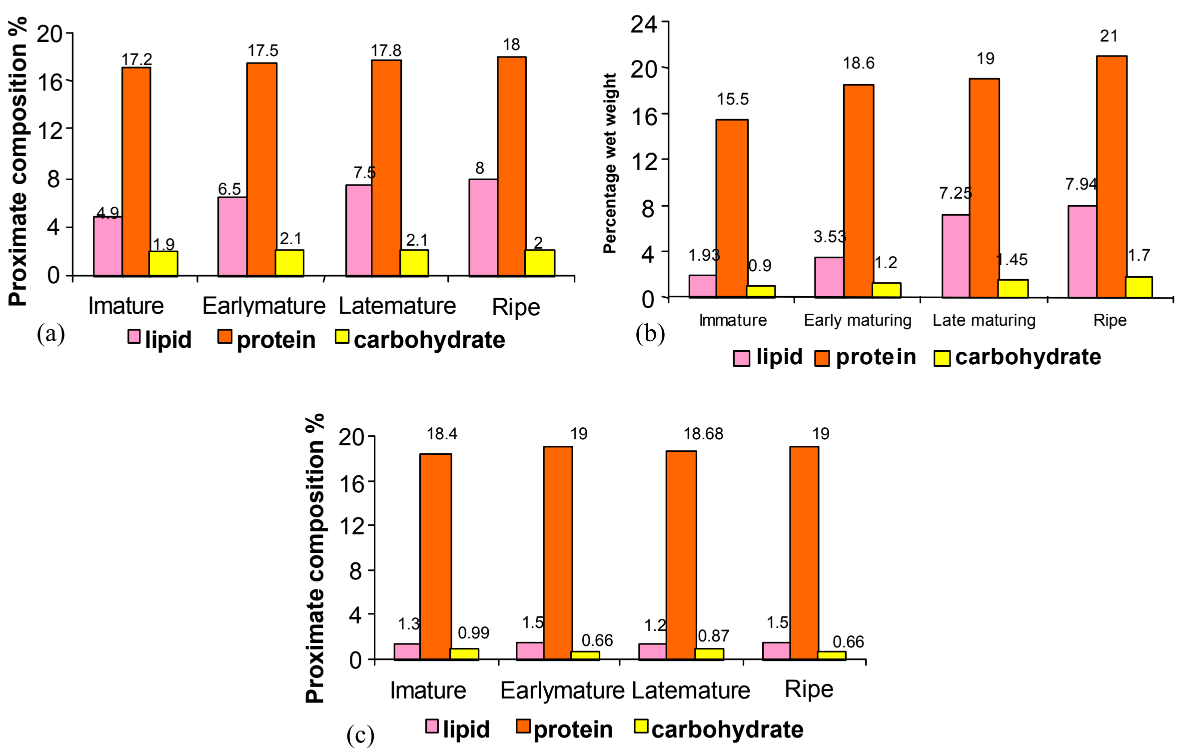

Fig. 1: Variation in the proximate composition in (a) midgut gland (b) ovary and (c) muscle of *Fenneropenaeus penicillatus* during different maturity stages

crustaceans [46]. Accumulation of the organic constituents and energy reserves in different species depend upon the food resources that fluctuate in their availability.

The ovary exhibited the pronounce changes in the proximate composition and has been found well in agreement with those reported in other penaeid species [47, 16, 19]. The proximate chemical composition in the ovary of *F. penicillatus* (Figure 1b) depicts an increase in the dry matter from 21.00 % (stage I) to 28.00 % (stage IV). The lipid content varied from 2.60 % (stage I) to 6.80 % (stage IV), showing a significant increasing trend during maturation whereas, protein increased from initial level of 15.90 % (stage I) to 18.40 % (stage IV). The increase in the protein content was not so significant in the midgut gland as that of the ovary during different stages of sexual maturation, this increase in the protein in the ovary may be attributed to several biochemical processes going on during maturation [48]. Pillay and Nair [46], Jeckel *et al.,* [49] and Castille and Lawrence [19] also reported an increase percentage of protein in the ovaries of *Metapenaeus affnis*, *Pleoticus muelleri*, *Penaeus aztecus and P. setiferus* throughout the sexual maturation. Carbohydrate was found to be lower in ovary as compared to midgut gland it fluctuated from 0.70 % (stage I) to 1.55% (stage II) with no significant changes during ovarian development however according to Harrison [50] carbohydrates play important roles in production of nucleic acids, as intermediates in production of energy and as component in ovarian pigments. Ash varied from 1.80 % (stage I) to 2.20 % (stage III and IV). The moisture content significantly decreased from 79.00 % (stage I) to 71.80 % (stage IV). Lpid and protein content increased and moisture decreased throughout maturation whereas, carbohydrates increased from immature to early maturing ovary and continue to decrease till the fully ripe stage. An inverse relationship exists between the moisture and other organic reserves, during the ovarian development [51, 49]. Jeckel *et al..*, [49] reported decrease of moisture content in *Pleoticus muelleri* with the increase of lipid and protein in the ovary during maturation. Variation in the biochemical composition with particular reference to the protein ,lipid and glycogen in ovary and midgut gland have also been reported for shrimp *Parapenaeosis hardwickiii* by Kulkarni and Nagabhushanam [18], the values reported are not comparable to the values of present study. They reported percentage of lipid, protein in very low concentration in relative to glycogen which was in abundance and that organic reserves were mobilized from the digestive gland to the ovary, whereas, in the present study organic reserves were not mobilized. Similar finding were observed for *P. aztecus* by Castillle and Lawrence [19]. The energy content increased significantly throughout ovarian development showing accumulation of energy. The main difference found between the proximate composition of ovary and MG was the higher percentage of lipid in MG than that of the ovary.

There was no significant increase observed in organic constituents of the muscle during the ovarian maturation and showed no correlation with the reproductive activity. The lipid content in muscle increased from 1.20 % (stage III) to 1.50 % (stage II and IV); the protein ranged from 18.40 % (stage I) to 19.0 % (stage II and IV); Carbohydrate content ranged between 0.66 % (stage II and IV) to 0.99 (stage I); ash content was 1.81 % at stage III to 1.86 % at stage I and IV, whereas, the moisture content followed an up and down trend with ranged from 77.45 % (stage I) to 76.99 % (stage IV). The increase in dry matter in muscle was almost negligible i.e., from 22.55 % (stage I) to 23.02 % (stage II). The energy value also followed up and down trend ranged between 394 kJ (stage I and Stage II) to 408 kJ (stage II and stage IV).

Despite the fact that the hepatopancreas has been considered as the universal organic reserve organ, not all decapods transport measurable lipid reserves from it to the ovaries [16,19]. During the present study, in spite of accumulation of energy reserves in the mid gut gland during ovarian development, no indication for utilization of energy reserves supplied from mid gut gland to the ovaries was found.

Therefore, shrimp species derived energy for reproduction directly from feed rather than using the stored energy. It may be concluded that effect of maturation process is not as pronounce in mid gut gland and muscle as that of ovary, where significant variations were found.

REFERENCES

1. Kazmi, Q.B., 2003. Marine Fauna of Pakistan. Series. Shrimps (Penaeoidea, Sergestoidea, Stenopodidea) Marine Reference Collection and Resource Center, Universty of Karachi, pp: 1-16.
2. Majid, A., 1988. Marine fisheries in Pakistan. In Proceedings of the “International Conference on Marine Sciences of the Arabian Sea” (Mary Thompson and Tirmizi, N.M. Editors), pp: 246-253.

3. Liao, I.C. and N.H. Chao, 1987. Experimental culture of three new candidates for shrimp farming in Taiwan, *Penaeus semisulcatus*, *P. brasiliensis* and *P. penicillatus*. Asian Fish. Sci., 1: 33-45.
4. Tirmizi, N.M., 1972. An illustrated key to the identification of Northern Arabian Sea penaeids. Pak. J. Zool., 4(2): 185-211.
5. Tirmizi, N.M. and Q. Bashir, 1973. Shore and offshore penaeid prawns of Northern Arabian Sea. Publ. Univ. Karachi, pp: 1-71.
6. Tirmizi, N.M. and B. Khan, 1970. A hand book on Pakistan marine prawn *Penaeus*. Publ. Univ. Karachi, pp: 1-52.
7. Hassan, H., 1989. Distribution and abundance of penaeid juveniles on Makran and Sind coast. Pak. J. Zool., 21(2): 147-152.
8. Hassan, H., 1992. Immigration of *Metapenaeus slebbingi, M. affinis* and *M. monoceros* juveniles in the creeks and backwaters near Karachi. Pak. J. Sci. Indus. Res., 35: 190-193.
9. Ayub, Z. and M. Ahmed, 1991. Species composition of Jaira and Kalri group of shrimp landings at Karachi Fish Harbour. Pak. J. Zool., 23(1): 45-50.
10. Sultana, R., 2000. Bionomics and population structure of the juvenile shrimps with particular reference to genus *Penaeus* from backwaters of Karachi coast: Ph.D thesis, University of Karachi, pp: 270.
11. Nisa, K., R. Fatima and R.B. Qadri, 1993. Chemical constituents and amino acid pattern of shrimp (*Penaeus merquiensis*) from Karachi coastal waters. Pak. J. Sci. Indus. Res., 36(3): 146-147.
12. Nisa, K., R Fatima, R.B. Qadri, A.K. Farooq and N. Mahmood, 1995. Proximate composition and macro- and micro-nutrients in finfish / shellfish from Karachi coastal waters. Tropic. Sci., 35: 156-160.
13. Nisa, K., R.B. Qadri, A. Touheed and A. Viqaruddin, 1996. Fatty acid profile of four marine species from Karachi coastal waters. J. Chem. Soc. Pak., 18(1): 44-47.
14. Nisa, K., R.B. Qadri, A. Touheed and A. Viqaruddin, 2001. Lipid and fatty Acid profile of ladyfish, dhother, sua, sole, aal and khagga from Karachi Coast. J. Chem. Soc. Pak., 23(3): 177-180.
15. Hendrickx, M.E., F. Páez-Osuna and H.M. Zazueta-Padilla, 1998. Biology and biochemical composition of the deep-water shrimp *Heterocarpus vicarius* Faxon (Crustacea: Decapoda: Caridea: Pandalidae) fromthe southeastern Gulf of California, Mexico. Bull. Mar. Sci., 63(2): 265-275.
16. Pillay, K.K. and N.B. Nair, 1973. Observation on the biochemical changes in the gonads and other organs of *Uca amulipes*. *Portunus pelagicus* and *Metapenaeus affinis* (Decapoda: Crustacea) during the reproduction cycle. Mar. Biol., 18: 167-198.
17. Read, G.H. and M.S. Caulton, 1980. Changes in mass and chemical composition during the molt cycle and ovarian development in immature and mature *Penaeus indicus* Milne Edwards. Comp Biochem Physio., 66A: 431-437.
18. Kulkarni, G.K. and R. Nagabhushanam, 1979. Mobilization of organic reserves during ovarian development in marine penaied prawn, *Parapenaeopsis hardwickii* (Miers). Aquaculture, 18: 373-377.
19. Castille, F.L. and A.L. Lawrence, 1989. Relationship between maturation and biochemical composition of the gonads and digestive glands of the shrimp *Panaeus aztecus* and *Penaeus setiferus* (L). J. Crust. Biol., 9: 202-211.
20. Gehring, W.R., 1974. Maturational changes in the ovarian lipid spectrum of the pink shrimp, *Penaeus duorarum duorarum* Burkenroad. Comp. Biochem. Physiol., 49A: 511-524.
21. Clarke, A., 1977. Seasonal variations in the total lipid content of *Chorismus Antarcticus* (Pfeffer) (Crustacea: Decapoda) at South Georgia. J. Exp. Mar. Biol., 27: 93-106.
22. Nagabhushnam, R. and U.M. Farooqui, 1982. Mobilization of protein glycogen and lipid during ovarian maturation in marine crab, *Scylla serrata* (Forskal). Indian J Mar. Sci., 11: 184-186.
23. Velieg, P., 1988. Proximate composition of New Zealand marine finfish and shellfish. FAO Document Repository, AE581/E., pp: 59.
24. Rosa, R. and M.L. Nunes, 2003. Biochemical composition of deep-sea decapod crustaceans with two different benthic life strategies off the Portuguese south coast. Deep Sea Res. Part 1: Oceanogr. Res., 50: 119-130.
25. Kandemir, Ş. and N. Polat, 2007. Seasonal variation of total lipid and total fatty acid in muscle and liver of rainbow trout (O*ncorhynchus mykiss* w., 1792) reared in Derbent Dam Lake. Turk J. Fish. Aquat. Sci., 7: 27-31.
26. Mazumder, M.S.A., M.M. Rahman, A.T.A. Ahmed, M. Begum and M.A. Hossain, 2008. Proximate composition of some small indigenous fish species in Bangladesh. Internat. J. Sustain. Crop Prod., 3(4): 18-23.

27. Ravichandran, S., G. Rameshkumar and A. Rosario Prince, 2009. Biochemical composition of shell and flesh of the indian white shrimp, *Penaeusindicus* (*H.milne* Edwards 1837) Am-Euras. J. Sci., Res., 4(3): 191-194.
28. Suryavanshi, U., RA. Sreepada, Z.A. Ansari, S. Nigam and S. Badesab, 2009. A study on biochemical changes in the penaeid shrimp, *Metapenaeus monoceros* (Fabricius) following exposure to sublethal doses of organochlorine pesticide (endosulfan). Chemosphere, 77(11): 1540-50. (Epub)
29. Spaargaren, D.H. and J.P. Haefner, 1994. Interactions of ovary and hepatopancreas during the reproductive cycle of *Crangon crangon* (L.) 2. biochemical relations. J. Crust. Biol., 14: 6-19.
30. Heldt, J.H., 1938. La reproduction chez Ies Crustaces Decapods des la famile des penaeides. Annals de l'.Institute. Oceanographique, 18: 31-206.
31. Hudinaga, M., 1942. Reproduction, development and rearing of *Penaeus japonicus*. Jap J. Zool., 10: 305-393.
32. King, J.E., 1948. A study of the reproductive organs of the common marine shrimp, *Penaeus setiferus* (Linnaeus). Biol. Bull. (Woods Hole), 94(3): 244-264.
33. Cumming, W.C., 1961. Maturation and spawning of the pink shrimp *Penaeus duorarum* Burkenroad. Trans. Am. Fish. Soc., 90(4): 462-468.
34. Subrahmanyam, C.B., 1963. A note on reproductive cycle of the prawn *Penaeus indicus* (M. Edwards) of the Madras coast. Curr. Sci., 32(4): 165-166.
35. Tuma, D.J., 1967. A description of the development of primary and secondary sexual characters in the banana prawn, *Penaeus merguiensis* de Man (Crustacea: Decapoda: Penaeinae). Aust. J. Mar Fresh Wat. Res., 18: 73-88.
36. Rao, P.V., 1967. Some observations on the biology of *Penaeus indicus* Milne Edwards and *P. monodon* Fabricius from the Chilka Lake. Indian J. Fish., 14: 251-270.
37. Duronslet, M., A.I. Yudin, R.S. Wheller and W.H. Clark, Jr., 1975. Light and fine structural of natural and artificially induced egg growth of penaeid shrimp. In Proceedings of the World Maricult Soc., 6: 105- 111.
38. Primavera, J.H., 1980. Studies on broodstock of supgo *Penaeus monodon* Fabricius and other penaeids at the SEAFDEC Aquaculture Department. Paper presented at the Internat Symp Coastal Aquacul, 12-18 January, Cochin, India, pp: 24.
39. Sultana, R., 1986. A Comparative study of the reproductive organs of the selected penaeid prawns (Penaeinae) of Pakistan. M.Phil. thesis, University of Karachi, pp: 170.
40. Ayub, Z. and M. Ahmed, 2002a. A description of the ovarian development stages of penaeid shrimps from the coast of Pakistan. Aquaculture Res., 33: 767-776.
41. Ayub, Z. and M. Ahmed, 2002b. Maturation and spawning of four commercially important penaeid shrimps of Pakistan. Ind J. Mar. Sci., 31(2): 119-124.
42. AOAC (Association of Officials Analytical Chemist), 1990. Official Methods of Analysis, 15th Ed. AOAC Arlington, VA.
43. Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, 1959. Colorimetric method of determination of sugars and related substances. Analytical Chemistry, 28: 350-356.
44. Folch, J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J.Biol .Chem., 266: 497-509.
45. FAO, 1989. Yield and nutritional value of the commercially more important fish species. FAO Fish Tech., 309: 1-187.
46. Gibson, R. and P.L. Barker, 1979. The decapods hepatopancreas. Oceanogr. Mar. Biol. Annu Rev., 17: 285-346.
47. Pillay, K.K. and N.B. Nair, 1971. The annual reproductive cycle of *Uca amulipes*. *Portunus* pelagicus and *Myetapenaeus affinis* (Decapoda: Crustacea) from the southwest coast of India. Mar. Biol., 11: 152-166.
48. Yehezkel, G., R. Chayoth, U. Abu, I. Khalaila and A. Sagi, 2000. High density lipoprotein associated with secondary vitellogenesis in the hemolymph of the cray fish *Cherax quadricarinatus*. Comp. Biochem. Physiol., 127B: 411-421.
49. Jackel, W.H., J.E.A. De Moreno and V.J. Moreno, 1989. Biochemical composition, lipid classes and fatty acids in the ovary of the shrimp *Pleoticus muelleri* Bate. Comp. Biochem. Physiol., 92B: 271-276.
50. Harrison, K.E., 1990. The role of nutrition in maturation, reproduction an embryonic development of decapod crustaceans: a review. J. Shellfish Res., 9: 1-28.
51. Love, R.M., 1970. The Chemical Biology of Fishes Vol 1 New York Academic Press. XVI, pp: 550.