Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 4, 2013

Research Article

PROTEIN ANALYSIS OF THE CRAB HAEMOLYMPH COLLECTED FROM THE TRASH

W. SYLVESTER FREDRICK, V. SADEESH KUMAR AND S. RAVICHANDRAN

CAS in Marine Biology, Faculty of Marine sciences, Annamalai University, Parangipettai 608502. Email: sylvesterfredy@gmail.com

Received: 12 July 2013, Revised and Accepted: 22 Aug 2013

ABSTRACT

Objectives: To estimate the protein analysis of the crab haemolymph collected from the trash of landing center Pazhayar.

Methods: four major crabs found in the trash were identified by Sakai method and taken for sexwise and organwise protein estimation by the Lowry et al method. The female crab haemolymph of *Dromia dehaani* shows the maximum protein content and hence it was taken for molecular weight estimation by SDS PAGE.

Results: The major protein content was found in the female crabs. The sexwise protein estimation shows that among the female crabs of the four species maximum protein profile was in female *Dromia dehaani* (11.97%) and minimum in female *Doclea ovis* (4.4%). In male crabs maximum protein content was also recorded in *D. dehaani* (10%) and minimum in *D.ovis* (3.8%). In sexwise molecular weight determination of *D.dehaani* male confirms that protein bands with molecular size ranging from 25kDa and in female show 43 kDa.

Conclusion: Hence the present study revealed that the haemolymph can be easily scrutinized without sacrifice. The haemolymph is found to be protein rich and can be investigated in peptide level studies.

Keywords: Crab Haemolymph, Protein estimation, Trash crabs, SDS PAGE, *Dromia dehaani.*

INTRODUCTION

Proteins are the most versatile macromolecules in living systems and serve crucial functions in all biological processes. They function as catalysts, transport and store other molecules such as oxygen, provide mechanical support and immune protection, generate movement, transmit nerve impulses, and control growth and differentiation [1]. Crabs, among numerous other invertebrates are considered as an essential shell fishery product [2].

Crabs are the best sources for food products including protein source for marine lives as well as for human. The nutritional quality of the crab proteins is very favourable when compared with other poultry animals. The previous literature contains conflicting reports on the effects of nutritional status upon blood protein concentrations in haemocyanin-containing species. The haemolymph proteins of marine invertebrates are unique in composition, as they do not contain immunoglobulin or albumin like proteins and the protein composition varies in relation to physiological and functional state of the animal. The relative contributions of haemocyte phenoloxidase and hemocyanin in the standard physiological ratio at which they occur in haemolymph have been investigated in the crab, *Cancer magister* [3]. The concentration of protein in the haemolymph shows wide difference among brachyuran crabs like *Carcinus maenas* and *Uca minax* [4] in which, 70-95% consists of the respiratory pigment haemocyanin [5]. Electrophoresis in stabilizing media has been widely used for accurate and rapid characterization of crustacean haemolymph proteins. The data concerning the blood protein composition lack uniformity, thus clearly demonstrate the occurrence of an important intraspecific variation of the haemolymph constituents. Sodium Dodecyl Sul phate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) is a common technique for separating proteins at good resolution.

The present study reports that four trash crab species of different families which were taken for protein analysis. The male and female crab's haemolymph, body tissue and hepatopancreas were analyzed for protein content. The crab which posses the maximum protein content was determined their molecular weight.

MATERIALS AND METHODS

Animal collection

Crabs were collected from the Pazhayar landing center. The crabs which are not for commercial purpose and non-edible crabs were separated from the trash. The trash was noticed by visiting the landing centre regularly. Samples of each species were collected from 4 to 5 heaps from single trawl. About 100 kg of heaps was randomly sampled every week.

Identification of crabs:

Four crab species from different families were observed as dominant species from the trash. The samples were brought to the laboratory, cleaned with a brush and identified using appropriate reference [6, 7, 8].

Collection of Haemolymph and organs

Haemolymph was obtained from the ventral part of the abdominal segment using a fine sterile 25 gauge needle and a 1ml syringe. To avoid haemocyte degranulation and coagulation, the haemolymph was collected in the presence of sodium citrate buffer, pH 4.6 (2:1, V/V) and an equal volume of physiological saline (0.85%, NaCl w/v) was added to it. To remove haemocytes from the haemolymph it was centrifuged at 2000 rpm for 15min. Supernatant was collected by aspiration and then stored at 4ºC until use. Each animal was subjected to a single bleed collections were being done at the time of use. Two different organs such as body tissue and hepatopancreas from the same sex which shows maximum protein profile were excised out, minced and homogenized in phosphate buffer saline (PBS, pH 7.2) using mortar and pestle and then centrifuged. The protein constituents of these organs were also analyzed along with haemolymph.

Estimation of haemolymph protein

From the four different families, major crab species were taken for the estimation of the protein concentration. The amount of protein present in haemolymph was measured by a spectrometer according to the method of Lowry *et al.*, [9] using a calibration curve prepared with different concentrations (0.1-0.5 mg/ml) of Bovine Serum Albumin (BSA) as standard. In a series of test tubes different aliquots of standard protein solution were pippeted out. All the test tubes were made up to 1ml with distilled water. In all the test tubes 5ml of reagent C was added and mixed well. The test tubes were allowed to stand for 10minutes then 0.5ml of Folin Ciocalteu reagent was added. The tubes were mixed immediately after each addition and kept at 37° C for 30 minutes. The colour developed was read at spectrometer (Spectro UK-VIS RS) in 650nm.

Molecular weight determination by SDS PAGE

SDS PAGE was performed according to the method of Laemmli, [10] using a slab gel with a linear gradient of 6-18% gel in the presence or absence of 1% β- mercarptoethanol. The gel was run at a constant

voltage of 250V for 2 hours. The molecular mass of the subunit of normal and challenged haemolymph was estimated by measuring its relative mobility in SDS-PAGE compared to those of the low molecular weight standards. A mixture of 6 proteins, from 14.4 to 94kDa, was used as protein markers. Protein bands were stained with Coomasie Blue (0.02% Coomassie brilliant blue R-250 in 50% methanol -7.5% of acetic acid). The haemolymph of the animal which shows maximum protein profile was subjected to SDS PAGE in sex wise. The molecular weight of the protein constituents of the body tissue, hepatopancreas and haemolymph was also analyzed.

RESULTS

Identification of crabs

Four different crab species were identified from the family Dromiidae- 1, Callapidae-2, Majidae-2 and Dorripidae-1 is listed in Table 2. One species in each family which is found to be huge in trash were taken for this study.

Table 1: Dominant crabs in the trash used for protein estimation

S. No.	Species name	Family name
1.	*Dromia dehaani* [11]	Dromiidae
2.	*Calappa calappa* [12]	Calappidae
3.	*Doclea ovis* (Herbest, 1788)[13]	Majidae
4.	*Dorripe dorsipus* [12]	Dorippidae

Comparison of haemolymph protein

The estimation of the crab's haemolymph protein was found to be high in *D.dehaani*-11.97 followed by *C.calappa, D.dorsipus* and *D.ovis.* The comparison of the protein content of the haemolymph of crabs is given in fig. 5.

Sex wise protein estimation

Among the female crabs of the four species maximum protein content was in female *D.dehaani* (11.97%) and minimum in female *D.ovis* (4.4%). In male crabs maximum protein content was recorded in *D. dehaani* (10%) and minimum in *D.ovis* (3.8%). The sexwise protein comparison of varied crabs was given in fig. 6.

Organ wise protein estimation

The female crabs of all the four species proteins in the body tissue hepatopancreas and haemolymph was varied in protein estimation. High level of protein content (11.97%) was recorded in the haemolymph of *D.dehaani*. Among the four experimental crabs observed hepatopancreas of *D.ovis* crab showed low content of protein (2%). The organ wise comparision of the protein content in various crabs is shown in fig. 7.

Molecular weight determination by SDS PAGE:

The haemolymph body tissue and hepatopancreas were used to estimate the molecular weight of proteins present in it by SDS – PAGE. Different molecular weight marker proteins were used. The maximum protein profile was found in the hemolymph of *D. dehaani* crabs and the molecular weight was determined. Two clear bands were detected in the gel that represents proteins of 43kDa from a female crab *D. dehaani* haemolymph and 25 kDa proteins of male haemolymph of *D. dehaani* (Fig. 8). Protein profile of body tissue and hepatopancreas was also analyzed by SDS PAGE. The staining gel revealed 43kDa band where the haemolymph concentration was found to be higher than other organs (Fig. 9).

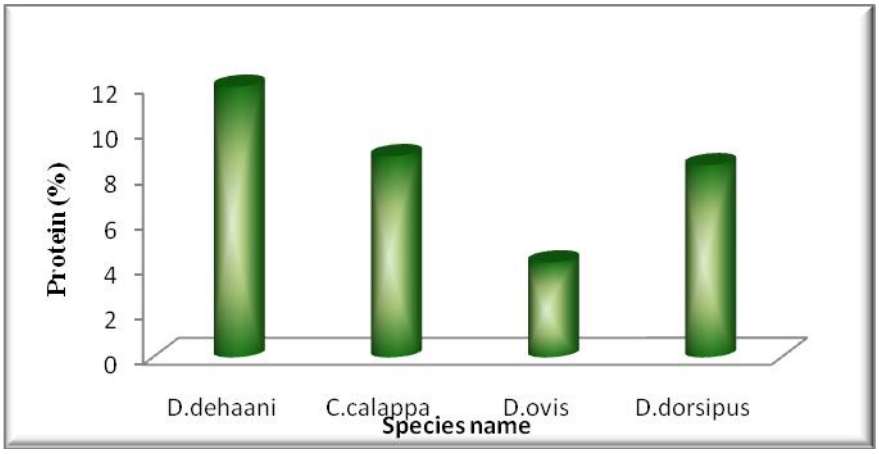

Fig. 1: Comparison of protein content in the haemolymph of crabs

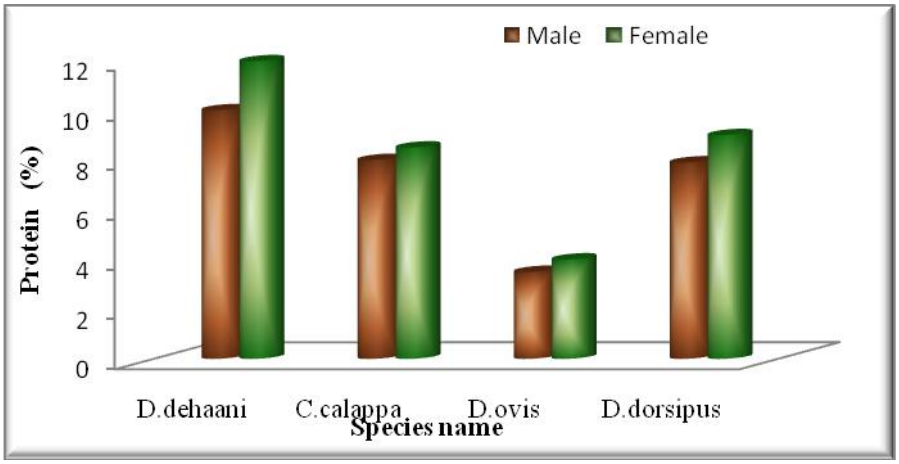

Fig. 2: Sexwise protein estimation in the haemolymph of crabs

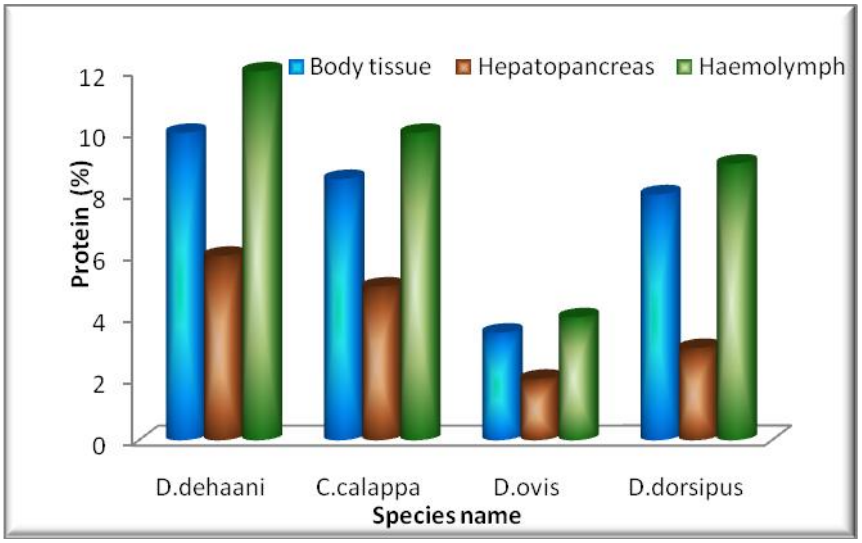

Fig. 3: Organwise protein comparisons of the four crabs.

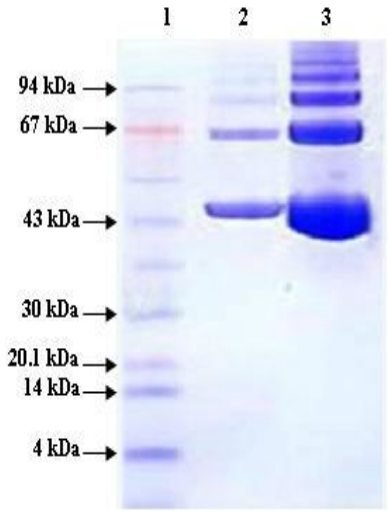

Fig. 4: Molecular weight of Haemolymph of male and female crabs Lane 1: Marker, 2: Male, 3: Female

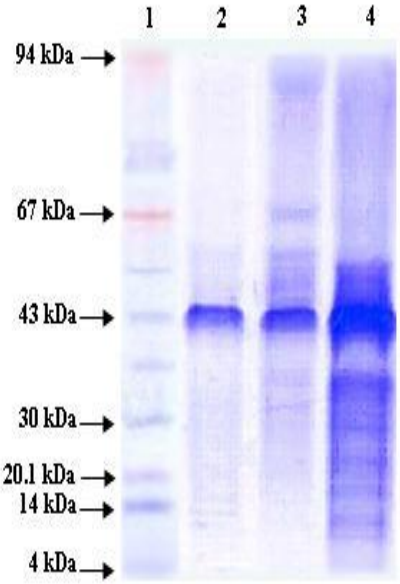

Fig. 5: Molecular weight of three different organs from the female crab *D.dehaani*. Lane 1: Marker, 2: Tissues, 3: Hepatopancreas and 4: Haemolymph

DISCUSSION

Crustacean haemolymph proteins vary with nutritional state. Blood proteins considerably changed during imposed fasting, qualitative studies of blood protein were mainly based on electrophoretic mobility of proteins. The recognition of the pathogens and parasites by the invertebrate immune system may involve soluble proteins present in the haemolymph as well as proteins localized at the surface of the haemocyte or other cells. The recognition stimulus and the secondary signals trigger signalling factors or induced transcription of genes for production of antimicrobial proteins [14]. The biochemical constituents of the animal are known to vary with season [15] size of the animal, stage of maturity, temperature and available of food etc. [16]. Protein is very much essential for the sustenance of the life and growth and hence it is present in largest quantity. It is the most prominent biochemical component of crustaceans from eggs to adults and is strikingly dominant in younger phases. The protein content in the crab was reported to be higher in hard shell crabs [17].

In the present study reports the protein content from the haemolymph; muscle tissue and hepatopancreas of the crabs were quantified. Among the crab investigated the haemolymph of *D.dehaani* was found to be maximum and the minimum protein content in the crab *D.ovis*. This result supports that the concentration of protein in the haemolymph shows wide interspecific variation among decapods crustaceans, ranging from as low as 28mg ml^{-1} in *C.maenas* [17] to as high as 222mg ml^{-1} in *Uca minax* [19] of the haemolymph protein, 70-95% consists of the respiratory pigment hemocyanin [20, 5]. Rameshkumar *et al*, [21] studied the haemolymph proteins from 15 species of brachyuran crabs among the species with maximum protein content of 10.97% was noticed in *Scylla serrata* and the minimum protein content of 2.30% in *Thalamita chaptali* crabs.

In the sexwise protein estimation of the present study the female crab's haemolymph consists of high protein content than male. The mature female crabs of all the four species and its protein content in haemolymph, body tissue and hepatopancreas were compared. The protein content is rich in *D.dehaani* and low content was noticed in *D.ovis* crabs. Variations in total haemolymph protein levels in crustaceans have been found in relation to the stages of the moult cycle [4], males and females, and females with and without egg-masses[22]. Serum protein levels in *C.maenas* [20] and *Homarus americanus* [23] have also been shown to decrease in response to starvation. The analysis of 41 individual serum protein concentrations in *Maia squinado* by Drach and Teissier [24] showed three to fourfold difference. The same individuals after a molt, despite an abrupt drop in protein concentration, showed the same range of individual variation. The results from the present study agree with the findings of above mentioned studies. The crab *Albunea symmista* was estimated for protein contents in haemolymph, ovary and hepatopancreas. The haemolymph protein level gradually raised from post molt to early premolt (2.7± 0.2 to 6.3 ± 0.2 mg/ml) and it get decreased in the late premolt due to water influx through new cuticle (6.3 ±0.2 to 4.3 ± 0.1 mg/ml). The protein content in the hepatopancreas shows both during reproductive molt cycle (29.5 ± 1.9 to 148.2 ± 1.8 µg/mg) and non-reproductive molt cycle (144.8±1.0 to 125.1 ±5. 2 µg/mg). The fluctuation of protein level occurs in haemolymph, and hepatopancreas during non-reproductive and reproductive molt cycle [25]. A similar increase in protein content of haemolymph during postmolt, intermolt, early premolt and its decrease during late premolt has been reported in *Penaeus indicus* [26]. The crab *Emerita asiatica* which breed continuously and repeatedly also show similar results to the present study in variation of haemolymph [27,28, 29].

In the molecular weight investigation from the crab *D. dehaani* when subjected after electrophoresis two clear bands were detected in the gel which represents the molecular weight of 43kDa in female crab and 25kDa in male crab. Likewise *Charybdis lucifera* was estimated for molecular weight in both sex results high in female with 45kDa and 25kDa in male crab [30]. Ismail *et al*. [31] reported three protein bands at 69KDa, 72KDa and 79KDa from *Tachypleus gigas*. The samples were further separated through polyethylene glycols to get ten sharp bands and eight faint bands from protein profiling of *Tachypleus gigas*. Similarly Yang *et al*., [32] reported low molecular weight proteins at 35 to 45KDa using western blotting analysis from ovary of *Portunus trituberculatus*. Okino *et al.* [33] also isolated similar molecular weight proteins were also isolated by from the horseshoe crab hemocytes. A haemolymph protein of 47kDa named hemolin, composed of repeated immunoglobulin domains, is thought to have a role in immune recognition and in modulation of defensive responses in *Hyalophora cecropia* and *Manduca sexta* [34]. In crustaceans, the hepatopancreas is generally regarded as a major lipid storage organ [35, 36]. The present study results supports for the hepatopancreas decrease level in the case of lipid concentration analysis in *Portunus sanguinolentus* of the haemolymph, ovary and hepatopancreas was studied in different stages. The lipid concentration of muscle and haemolymph was lower than that of ovary and hepatopancreas and it did not change markedly during the ovarian development. On the other hand, the lipid concentration of ovary and hepatopancreas varied markedly with that of ovarian maturation [37].

However the haemolymph concentration is more and this might be due to the utilization of the protein for new cuticle formation. The haemolymph protein level plays a vital role in the reproduction. The proteins involved in the haemolymph are necessarily sequestered into the ovary for its maturation. The hepatopancreas doesn't show any correlation with tissue and haemolymph protein. This is mainly due to that hepatopancreas is partly involved in reproduction mainly in carbohydrate and lipid levels in the synthetic site together with their utilization during different physiological events [25].

Thus the protein concentration from the haemolymph of four crabs was estimated. The maximum protein content is shown in female crab and the molecular weight of the female haemolymph in *D.dehaani* was revealed to be 43kDa. Further, the low molecular weight protein is taken for purification, functional characterization and the recognition of peptide can studied.

REFERENCES

1. Berg JM, Tymoczko JL, Stryer L *Biochemistry*. 5th edition. New York: W H Freeman 2002.
2. Nalan G, Dlua K, Yerlikayaa P Determination of Proximate Composition and Mineral Contents of Blue Crab (*Callinectes sapidus*) and Swim Crab (*Portunus pelagicus*) Caught off the Gulf of Antalya. Food Chem 2003; 80: 495-498.
3. Terwilliger NB, Dangott L, Ryan M Cryptocyanin, a crustacean molting protein: Evolutionary link with arthropod hemocyanins and insect hexamerins. Proc. Natl. Acad. Sci U. S. A. 1998; 96(5): 2013-2018.
4. Depeledge MH Bjerregard P Haemolymph Protein composition and copper levels in decapods crustaceans. Helgol. Meeresenters 1989; 43: 207-223.
5. Zatta P Zinc transport in the haemolymph of the shore crab *Carcinus maenas*. J. Mar. Biol. Assess U.K. 1984; 64: 801-807
6. Sakai T Crabs of Japan and the adjancent Seas. Kodansha Tokyo, Japan. 1976; 773 pp.
7. Sakai K, Turkay M, Yang SL Revision of the *Helice*/ *Chasmagnathus* complex (Crustacea: Decapoda: Brachyura). Abh. Senckenberg. Naturforsch. Ges 2006; 565: 1-76.
8. Sethuramalingam S, Ajmal Khan S Brachyuran crabs of Parangipettai coast. CAS in Marine Biology, Annamalai University, Parangipettai, India. 1991.
9. Lowry Oh, Rosebrough Nj, Farr Al, Randall Rj Protein measurement with the Folin phenol reagent. J. Biol. Chem 1951; 193(1): 265-275.
10. Laemmli UK Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. Nature 1970; 227: 680–685.
11. Rathbun MJ An analysis of *Dromia dormia* (Linnaeus). Proceedings of the biological Society of Washington, 1923; 36: 65-70.
12. Linnaeus C Systema Naturae per Regna tria Naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. (Animalia) 1758; 1(2): 1-823.

13. Herbst JFW Versuch einer Naturgeschichte der Krabben und Krebse, nebst einer systematischen Beschreibung ihrer verschiedenen Arten 1782-1804; 1-3: 515 pp., 62 pls.
14. Gillespie JP, Kanost MR Trenczek T Biological mediators of insect immunity. Ann. Rev. Entomol 1997; 42: 611–643.
15. Akbar Z, Qasim R Siddiqui PJA Seasonal variation in biochemical composition of edible crab, *Portunus pelagicus* (Linneaus). J. Islamic Acade. Sci 1988; 1(2): 127-133.
16. Soundarapandian P, Ananthan G Effect of unilateral eyestalk ablation and diets on the biochemical composition of commercially important juveniles of *Macrobrachium malcomsonii* (H. Milne Edwards). Intern. J. Zool. Resear 2008; 4(2): 106-112.
17. Sudhakar M, Manivannan K, Soundrapandian P Nutritive value of hard and soft shell crabs of *Portunus sanguinolentus* (Herbst). Inter. J. Ani. and Veterin. Adv 2009; 1(2): 44-48.
18. Gilles R Effect of osmotic stresses on the protein concentration and a pattern of *Eriocheir sinensis* blood. Comp. Biochem. Physiol 1977; 56: 109-114.
19. Pequeux A, Vallota AC, Gilles R Blood proteins as related to osmoregulation in crustacean. Comp. Biochem. Physio 1979; 64: 433-435.
20. Uglow RF Haemolymph protein concentrations in portunid crabs-II. The effect of imposed fasting on *Carcinus maenas*. Comp. Biochem. Physiol 1969; 31: 959-967.
21. Rameshkumar G, Ravichandran S, Kaliyavarathan G, Ajithkumar TT Antimicrobial peptide from the crab, *Thalamita crenata* (Latreille, 1829). World J. Fish and Marine Sci 2009; 1(2): 74-79.
22. Horn EC, Kerr MS Hemolymph protein and copper concentration of adult blue crab (Callinectes sapidus Rathbun). Biol. Bull (Woods Hole) 1963; 125(3): 499-507.
23. Stewart JE, Cornick JW, Dingle JR An electronic method for counting lobster (*Homarus americanus* -Milne Edwards) hemocytes and influence of diet on hemocyte numbers and haemolymph proteins. Can. J. Zool 1967; 45: 291-304.
24. Drach P, Teissier G Mue et protidemie chez les crabs. C.R. Soc. Biol 1939; 131: 1199-1201.
25. Kanagalakshmi K Fluctuation of protein level in Haemolymph, ovary and hepatopancreas during non-reproductive and reproductive Molt Cycle of *Albunea symmista*. J. Resear. Biology 2011; 2: 68-72.
26. Read GHL, Caulton MS Changes in mass and chemical composition during the moult cycle and ovarian development in immature and mature *Penaeus indicus* Milne edwards. Comp. Biochem. Physiol 1979; 66: 431-437.
27. Subramoniam T Continuous breeding in the tropical anomuran crab, *Emerita asiatica* (Milne Edwards). J. Exp. Mar. Biol. Ecol 1977; 65: 259-268.
28. Subramoniam T Some aspects of reproductive ecology of a mole crab, *Emerita asiatica* (Milne Edwards). J.Exp. Mar Biol. Ecol 1979; 65: 259-268.
29. Gunamalai V Synchronization of molting and oogenic cycles in a continuously breeding population of the sand crab *Emerita asiatica* on the madras coast, South India. J. Crust. Biol 2002; 22: 377-389.
30. Rameshkumar G, Ravichandran S, Arvaindhan T Antimicrobial proteins from the crab *Charybdis lucifera* (Fabricus, 1798). Mid-East. J. Sci. Res 2009b; 4(1): 40-43.
31. Ismail N, Sarijan S Phylogenetic Inference from 18S rRNA Gene Sequences of Horseshoe Crabs, *Tachypleus gigas* between Tanjung Dawai, Kedah and Cherating, Pahang, Peninsular Malaysia. Intern. J. Agri. Biolo. Sciences 2010; 1: 2.
32. Yang F, Huna TX, Zhong D Miin, Yang WJ Molecular characterization and expression analysis of vitellogenin in the marine crab *Portunus trituberculatus*. CBP Biochem. Mole. Boil 2005; 142(4): 456-464.
33. Okino N, Kawabata S, Saito T, Hirata M, Takagi T, Iwanaga S Purification, characterization and cDNA cloning of a 27-kDa lectin (L10) from horseshoe crab hemocytes. J. Biol. Chem 1995; 270: 31008-31015.
34. Jiang H, Kanost MR Characterization and functional analysis of 12 naturally occurring reactive site variants of serpin-1 from *Manduca sexta*. J. Biol. Chem 1997; 272: 1082-1087.
35. Ando T, Kanazawa A, Teshima S Variation in the lipids of tissues during the molting cycle of prawn. Bull. Jpn. Soc. Sci. Fish 1977; 43(12): 1445-1449.
36. Teshima S, Kanazawa A Variation in lipid composition during the ovarian maturation of the prawn. Bull. Jpn. Soc. Sci. Fish 1983; 49: 957-962.
37. Ravichandran S, Rameshkumar G, Velankanni S, Ajithkumar TT Variation in Lipid concentration of the crab *Portunus sanguinolentus* at different developmental Stages. Middle-East J. Scient. Resear 2009; 4(3): 175-179.