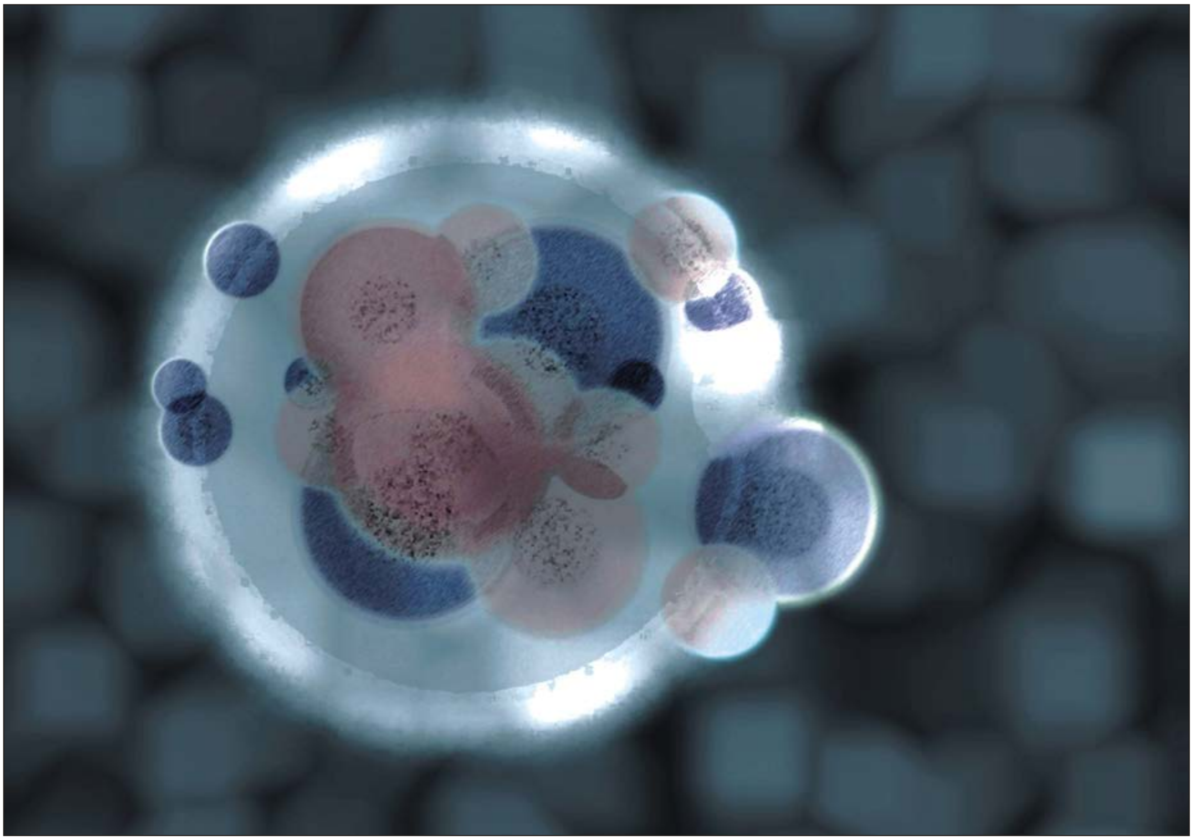

Journal of Biological Sciences

ISSN 1727-3048

Journal of Biological Sciences 11 (2): 203-209, 2011
ISSN 1727-3048 / DOI: 10.3923/jbs.2011.203.209

Influence of the Eyestalk Hormones on the Metabolism and Ionic Regulation of the Crab *Portunus pelagicus* (Lineaus, 1857)

Bilal Ahmad Bhat, S. Ravichandran and Sartaj Ahmad Allayie
Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai 608502, Tamil Nadu, India

Abstract: Biochemical studies are very important from the nutritional point of view. The biochemical constituents in animals are known to vary with season, size of the animal, stage of maturity, temperature and availability of food also to elucidate the osmoregulatory ion concentration. The present study quantified the nutritive value of sea food and the ionic regulation which is good measure for leading a fruitful aquaculture. So for methods used for the detection of sodium and chlorideion concentrations are Elico digital flame photometer. Biochemical composition of hemolymph and different tissues was carried out and it has been observed that more protein content were found in eyestalk extract injected female crabs (64.0%) while as less percentage was found in normal female crab (10.66%) and lipids were found more in injected female and ablated male (16.8 mg mL^{-1}) while it is very low in normal male crab (12.6 mg mL^{-1}). Carbohydrate percentage was found more in ablated male (7.6%) and less in eyestalk extract injected male (3.3%). The variations of the sodium and chloride ionic level in the hemolymph were studied by comparing the levels in the experimental animals with that of control animals. In the ablated animals of male crabs, the hemolymph showed decreased sodiumionic level (428 milliequivalent L^{-1}) when compared to control animals while as in female it showed increase in the Na^{+} ion concentration (454 milliequivalent L^{-1}). However, eyestalk extract injected animals of both sexes at the final stage showed a much increase in the sodium ionic level when compared to control and ablated animals.

Key words: Hemolymph, protein, lipid, carbohydrate, sodium ions, chlorideions

INTRODUCTION

Eyestalk ablation is a frequently adopted procedure for induced maturation of gonads. Unilateral eyestalk ablation has been employed to induce both ovarian maturation and spawning with varying success in many species (Zaib-Un-Nisa, 2001). Unilateral eyestalk ablation can also be used to shorten the moult interval and to stimulate gonad development in shrimps (Lin *et al*., 2001).

Marine invertebrates are widely used as a source of food and feed supplements throughout the world. Among the edible marine crustaceans of India, crabs rank third by virtue of their importance as an esteemed gourmet and the value of fishery they support while the first and second place are occupied by shrimps and lobster, respectively. In Indian scenario, the consumers mostly prefer bigger crabs viz., *Scylla serrata* and *S.tranquebarica* and they purposely neglect swimming crabs. Even though the swimming crabs viz., *P. sanguinolentus* and *P. pelagicus* are available throughout the year in Indian coasts (Samuel *et al*., 2004). Ravichandran *et al.* (2009) reported variation in fatty acid composition of the crab *Portunus sanguinolentus* in different developmental stages. Ravichandran and Kannupandi (2004) have investigated variation in biochemical composition of the various brachyuran crabs at different developmental stages. De Freitas Antunes *et al.* (2010) have demonstrated the seasonal variations in the biochemical composition and reproductive cycle of the ghost crab *Ocypode quadrata*. Sainath and Reddy (2010) have determined the melatonergic regulation of hemolymph sugar levels in the freshwater edible crab, *Oziotelphusa senex senex*.

The mechanisms of osmotic and ionic regulation have been demonstrated to be under neuroendocrine control. The importance of the eyestalk ganglionic system in the regulation of water and ion movement has also been demonstrated in a variety of crustaceans and it was also concluded that eyestalk removal might initiate a lowering of hemolymph sodium due to increased sodium eflux and result in an increase of sodium permeability in crustaceans (Davis and Hagadorn, 1982). Moreover, Charmantier *et al.* (1984) reported that eyestalk neurohormonal factor may regulate hemolymph osmosis, sodium and chloride concentration in

Corresponding Author: Bilal Ahmad Bhat, Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai 608502, Tamil Nadu, India Tel: + 91-4144-243223, 243533

Homarus americanus. In addition to the above-described eyestalk ablation also lowers the sodium concentration in hemolymph McNamara *et al.* (1990). Although biochemical composition of this crab *P. pelagicus* has been carried out by many workers but till now no such studies has been carried out regarding the neuroendocrine influence of eyestalk hormones on biochemical composition and ionic regulation. Hence an attempt was made to carry out the preliminary study to validate the proximate composition of hemolymph and sodium chloride ion regulation which is influencing the crab with reference to the eyestalk ablation and eyestalk extract injection.

MATERIALS AND METHODS

The crabs for the current study were collected from neritic zone of Vellar estuary during the period August-September 2010. After collected from the net the inter-moult crabs were taken for eyestalk related experimental studies. Other than the inter-moult stage crabs were investigated for proximate composition. The crabs were segregated as male and female in different tubs at the laboratory. The optimum environmental parameters were maintained during experimental period (Salinity 33-35 ppt; pH 7.5-8.0; Temperature 28-31°C). Crabs were fed with fresh fishes, bivalves and some of the crustacean like squilla and hermit crab muscles twice a day. The water was being exchanged after two days regularly in morning hours and left over feed and fecal matters were removed.

Eyestalk ablation and extracts preparation: Bilateral extirpation was carried out by cutting the both eyestalks of experimental crabs below the eye stalk using Pre-Sterilized scissors and forceps. The wound was cauterized with a hot blunt needle to prevent the loss of hemolymph.

Eyestalks were isolated from crabs which were already have been kept under the ice to get anesthetize. For about five minutes the crabs were kept in the ice. After anesthetizing the crabs eyestalks were removed by cutting through the arthrodial membrane at the base of each eyestalk using clean dissecting scissors and forceps. The isolated eyestalks, exoskeleton intact were stored at -20°C. The exoskeletons of eyestalks were removed with dissecting instruments. The soft tissues isolated were homogenized and centrifuged at 15,000 rpm for about 10 min at 2°C. The supernatant was collected in a pre-chilled micro centrifuge tube and homogenate re-extracted as before. The final supernatant containing the eyestalk extract was transferred into cold micro centrifuge tubes and stored at -20°C until required.

Injection: A single dose of gland extracts of the isolated supernatants were injected into the animal body through the arthrodial membrane at the base of the coxa of the third pair of walking legs to observe some of the physiological changes.

Tissue and hemolymph collection: Different tissues from the crabs were taken for biochemical composition in different stages. Crabs selected for extraction of tissues were first anesthetized by submergence into saline water containing ice. The tissues/organs were then removed from the crabs by cutting along the dorsal surface just below the cuticle to ensure no perforation of tissue occurred. The incision was then carefully opened and muscle tissues, heart, ovary, hepatopancreas and shell tissues were removed. Hemolymph was collected from the arthrodial membrane covering the articulated base of the walking legs. The area was swabbed or flushed with 70% ethanol to sterilize the surface and a 25 gauge sterile hypodermic needle and syringe was used to extract around 1 mL of hemolymph from each crab. The hemolymph was collected in sterilized pre-chilled test tubes contain anticoagulant and stored at -20°C until required.

Determination of total protein, carbohydrate and lipid contents: The protein, carbohydrate and lipid contents were estimated by adopting the standard methods of Raymont *et al.* (1964), DuBois *et al.* (1956) and Folch *et al.* (1957), respectively.

Sodium and chlorine ion detection from the hemolymph: Sodium and chlorine ion concentration of control, eyestalk ablated and eyestalk extract injected was determined by following methods, respectively; Sodium concentration was determined using Elico digital flame photometer and chloride concentration in the hemolymph was determined calorimetrically by the method of Schoenfeld and Lewellen (1964).

RESULTS

Influence of eyestalk ablation and eyestalk extract injection on biochemical composition: In the present study, the effect of eyestalk ablation and eyestalk extract injection in the crab *P. pelagicus* and its physiological response over it is well monitored. The biochemical investigation of the crab tissues after the experimental set up is given in Table 1 and 2.

Protein: The protein content of different tissues in different stages e.g., ovary (stage 1 and 2), Hepatopancreas (Moulting and Intermoult), heart

Table 1: Protein, lipid and carbohydrate content present in the different tissues of crab (*Portunus pelagicus*)

Name of the sample	Protein content (%)	Lipid content mg g^{-1}	Carbohydrate content (%)
Ovary (stage 1) white mass	1.32	10.00	1.20
Ovary (stage 2) orange mass	3.63	10.10	1.10
Hepatopancreas (inter-moult)	0.53	8.70	0.56
Hepatopancreas (moulting)	2.33	10.00	1.30
Heart of the female (unbrood)	1.11	2.70	1.10
Heart of the female (brood)	5.13	3.20	1.10
Yellow egg mass (brood)	10.73	13.00	1.10
Brown egg mass (brood)	7.80	11.00	1.10
Shell epidermis of normal crab	5.74	0.90	1.30
Shell epidermis moulting crab	8.00	0.50	1.35
Shell during moulting	2.73	0.00	1.30
Shell during intermoult	0.83	0.01	0.30

Table 2: Protein, lipid and carbohydrate content present in the hemolymph of crab (*Portunus pelagicus*)

Name of the sample	Protein content (%)	Lipid content mg mL^{-1}	Carbohydrate content (%)
Hemolymph of normal crab (male)	38.50	12.6	6.2
Hemolymph of normal crab (female)	10.66	14.5	4.5
Hemolymph of injected crab (male)	12.15	13.7	3.3
Hemolymph of injected crab (female)	64.00	16.8	6.5
Hemolymph of ablated crab (male)	33.04	16.8	7.6
Hemolymph of ablated crab (female)	31.90	16.5	5.6

(Brood and Unbrood), yellow-brown egg mass, shell epidermis (Moulting and Intermoult), shell (Moulting and Intermoult) and hemolymph of control, ablated E-injected crabs are given in Table 1 and 2. The result showed that the protein content is higher in yellow egg mass (10.73%) and lowest in hepatopancreas intermolt (0.53%) (Table 1). The present study clearly showed that eyestalk ablation had increased the protein contents in both sexes.

Lipid: The lipid content of hemolymph in eyestalk injected female crab and eyestalk ablated male animals was observed much higher (16.8 mg mL^{-1}) as compared to others normal animals (Table 2).

The lipid content of different tissues in different stages was recorded maximum in ovary (Orange, colour) stage 2 and minimum in shell epidermis moulting crab (Table 1). The result also showed that the lipid content of hemolymph in eyestalk injected crabs in both sexes were much higher than normal and ablated animals. Hence it was observed from the present study that eyestalk injections has increased protein content more in females.

Carbohydrate: The carbohydrate content of different tissues in different stages e.g., ovary (stage 1 and 2), hepatopancrease (Moulting and Intermoult), heart (Brood and Unbrood), yellow and brown egg mass, shell epidermis (Moulting and Intermoult), shell (Moulting and Intermoult) and hemolymph of control, ablated E-injected crabs were also observed (Table 1 and 2). The result showed that the carbohydrate content is highest in shell

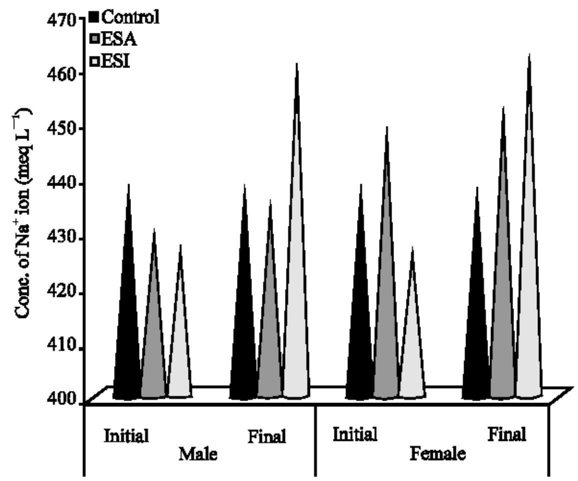

Fig. 1: Comparison of sodium ion concentration between male and female of *P. pelagicus*

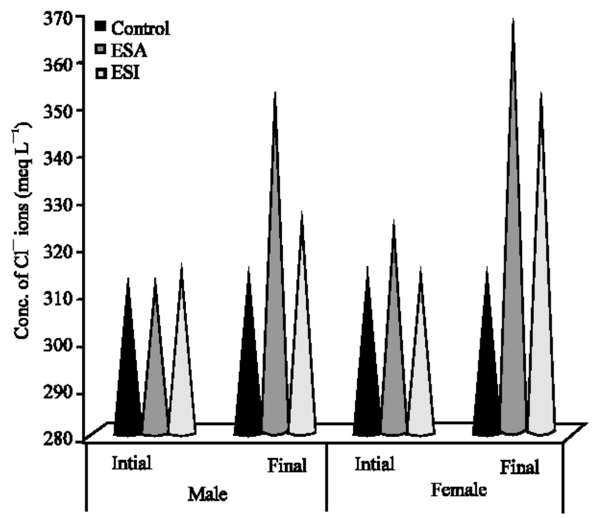

Fig. 2: Comparison of chlorine ion concentration between male and female of *P. pelagicus* (ESA: Eyestalk ablation, ESI: Eyestalk injection)

epidermis during moulting stage of the crab (1.35%) and lowest in Shell during inter-moult (0.3%) (Table 1). The present study also showed that eyestalk ablation has increased the carbohydrate contents of hemolymph in both sexes (Table 2).

Influence on sodium and chlorine ion: The variations of the sodium and chloride ionic level in the hemolymph were studied by comparing the ionic levels of the experimental animals with that of control animals and it is shown in Fig. 1 and 2. In the ablated animals of male crabs, the hemolymph showed decreased sodium ionic level when compared to control animals while as in female it showed increase in the Na^+ ion concentration. However, eyestalk extract injected animals of both sexes at the final stage showed a much increase in the sodium ionic level when compared to control and ablated animals. Similarly chlorine level in the hemolymph of ablated male crabs showed no difference in the beginning which increased to a high level after fourth hour. However, the level of chlorine showed much more increase in the injected once at final stage when compared to normal. While as in ablated and injected female animals, it has shown increased level of chlorine from beginning up to the fourth hour. So it was observed that the maximum level of both Na^+ and Cl^- ions was found high in the injected crabs.

DISCUSSION

Biochemical studies are very important from the nutritional point of view. The biochemical constituents in animals are known to vary with season, size of the animal, stage of maturity, temperature and availability of food etc. The eyestalk hormones have regulative (positive) influence on carbohydrate, nitrogen and lipid metabolism in crustaceans (Highnam and Hill, 1979) which were found to vary with times and species (Vernberg and Vernberg, 1974; Madyasthan and Rengnekhar, 1976).

In the present study, the animals were classified based on the maturity of ovary; moulting and inter-moult stages were taken. An attempt has been made to study the quantitative variation in the biochemical constituents of hepatopancreas, hard and soft shell in normal and moulting stages. The biochemical composition of ovary and egg mass were also evaluated. The energy metabolism of crustaceans is characterized by high intra- and inter-specific variability which makes it difficult to determine a standard metabolic profile Oliveira *et al.* (2003). Most species reduce their metabolic rate and deplete protein, glycogen and lipid reserves during nutritional stress (Vinagre and Da Silva, 1992; Oliveira *et al.*, 2004; Comoglio *et al.*, 2005).

The findings of he present study shows the protein content was higher in yellow egg mass (brood) and in the hemolymph of injected female and male crabs, respectively. Lipid content was higher in the brown egg mass (brood) and in the hemolymph of injected female and male crabs, respectively. Similarly in the present study, carbohydrate content was found high in shell epidermis of moulting crab and in the hemolymph of ablated male. So our results are in agreement with the results of Soundarapandian and Ananthan (2008). But disagreed the statement/result that eyestalk ablation does not have any effect on carbohydrate content as in present study eyestalk ablated animal are having more percentage of carbohydrates as compared to other injected and control crabs in *P. pelagicus*. However, reports from the study of Surendranath *et al.* (1992) are in agreement with the present study. Present study also disagreed with the results of Koshio *et al.* (1992) in respect to proteins and lipids, because of his statement that neither unilateral eyestalk ablation nor feeding frequency affected the contents of the protein, lipids. However, Soundarapandian and Singh (2008) reported that the protein, lipid and carbohydrate content of the eggs of *P. pelagicus*. Protein content was significantly higher in the hemolymph of eyestalk injected animals than those of eyestalk ablated and normal crabs hence present study is disagreed with the reports of Sheen and D'Abramo (1991) and Shah (1995) as their results are showing that ablation increases the protein content.

A significant finding of the present study was that in control crab *P. pelagicus* the biochemical compositions of different tissue vary considerably. However, it is high in egg masses and epidermis of mature crabs. As in other studies, this work documents an increase in measures of ovary lipid with ovarian maturation (Castille and Lawrence, 1989; Mourente and Rodriguez, 1991; Mourente *et al.*, 1994; Tuck *et al.*, 1997; Wen *et al.*, 2001; Rosa and Nunes, 2002).

The increase in biochemical contents in tissues may be due to the transfer of more biochemical components from muscles towards these tissues during maturity. Further, the increase in biochemical composition of hemolymph in injected crabs may be due to some eyestalk hormones of these animals which may induce the metabolism, hence the present study is in agreement with the findings of Zafar *et al.* (2004) and Murugesan *et al.* (2008) who reported that ablation, increased the biochemical composition of crustaceans.

Crustaceans are characterized by a wide range of osmoregulatory powers which are developed based on the environment to which the animals are adapted. The capacity to regulate the ions aids the prawns in the successful completion of their life history in the wide-differing aqua-regions, viz., the sea and the estuary. The ionic ability seems to be a purely adaptive feature that may change markedly during development according to environmental needs (Dall, 1981).

The most important ions involved in the maintenance of osmotic balance are the sodium and chloride. The exchange of these ions results from passive diffusion in an isosmotic medium as has been shown in *Carcinus maenas* (Zanders, 1980), *Hemigrapsus* sp. and *Pachygrapsus crassipes* (Rudy, 1966). In the present study the ionic influxes is highly equlibiriated with reference to the eye stalk ablation and extract injection. Chloride ion is an osmotically important solute in the hemolymph of crustaceans. It has been proved earlier that the ionic movements are under a primary controller-hormonal action. Also, that the crustacean eyestalk possesses a hemolymph-chloride-regulating factor has been confirmed in a number of freshwater crustaceans. It was also reported that the brain and thoracic ganglion of this animal have a hemolymph- chloride-increasing factor. Nan *et al.* (2004) reported that osmolarity, potassium and calcium concentration were high bilaterally ablated shrimps. Whereas the concentration of potassium and calcium were found low in unilaterally eyestalk ablated and control animals. However, the sodium and chloride concentration were found very low also decreased further with the increasing treatment time. He further observed that eyestalk ablation of shrimps causes changes in the osmotic and ionic regulation, and also effects the Na^+ ions of the gills.

In shrimps Cl^-, Na^+ and K+ levels were lower during the postmoult and were higher during intermolt and early premolt stages is considered to be associated with water uptake at the time of molting (Cheng *et al.*, 2002).

The ionic regulation in the marine crustaceans under hormonal influence, however, revealed different from that of the freshwater forms. In crustacean dopamine is also involved in osmoregulatory process as reviewed by Morris (2001) and Tierney *et al.* (2003). The same mechanism has been observed in the present study with high ionic influx in the case of eye stalk extract injected crab when compared to control crab. At the body surfaces it would decrease the outward permeability of the epithelial cells while at the same time promoting the active uptake of sodium across the gills by stimulating a Na^+, K^+ activated ATPase such as was found in the gills of the terrestrial crab, *Cardiosoma quanhumi,* by Quinn and Lane (1966). Eckhardt *et al.* (1995) have observed that CHH may be an important osmoregulatory neuropeptide, a view which is reinforced considering previous studies that have shown that perfusion of gills of *P. marmoratus* sinus gland extracts (Mr>5 kDa) rapidly increase Na^+ influx and TEP. At the body surfaces it would decrease the outward permeability of the epithelial cells while at the same time promoting the active uptake of sodium across the gills by stimulating a Na^+-K^+ activated ATPase such as was found in the gills of the terrestrial crab, *Cardiosom quanhum*, (Quinn and Lane, 1966). Moreover, the $DPhe^3$ isoform of *Astacus leptodactylus* CHH increases Na^+ content of the hemolymph in postmoult crayfish, that had previously been eyestalk ablated (Serrano *et al.*, 2003). Present research is having concurrence with (Eckhardt *et al.*, 1995; Spanings-Pierrot *et al.*, 2000). *Pachygrapsus marmoratus* show that Sinus Gland (SG) extracts significantly increase Na^+ and Trans Epithelial Potential (TEP) in posterior (ionoregulatory gills) in this crab.

CONCLUSION

In conclusion, the present study documented that eyestalk ablation and extract injection clearly influence the metabolism of proteins, lipids and carbohydrates and also influences the ionic regulations of the treated animals (crabs) to a great extent. Hence in the present study it has been observed that eyestalk extract increases the concentration of sodium and chlorine ions as a whole to a great extent which may be helpful in understanding the neuroendocrine effect of eyestalk as well may be helpful to improve the aquaculture to the leading. Because no reliable information is available about the mechanism of eyestalk hormones in ionic regulation. The sodium controlling hormones may be acting on the body surfaces and excretory organs. Further investigation is warranted to show how the hormones deploy the ion regulating systems.

REFERENCES

Castille, F.L. and L.A. Lawrence, 1989. Relationship between maturation and biochemical composition of gonads and digestive glands of shrimps *Penaeus aztetus* and *Penaeus setiferus*. J. Crust. Biol., 91: 202-211.

Charmantier, G., M. Charmantier-Daures and D.E. Aiken, 1984. Neuroendocrine control of hydromineral regulation in the American lobster, *Homarus americanus* H. Milne Edwards, 1837 (Crustacea, Decapoda). 1. Juveniles. Genet. Comp. Endocrin, 54: 8-19.

Cheng, W., C.H. Liu, D.F. Yan and J.C. Chen, 2002. Hemolymph oxyhemocyanin, protein, osmolarity and electrolyte levels of white leg shrimp *Litopenaeus vannamei* in relation to size and molt stage. Aquaculture, 211: 325-339.

Comoglio, L., L. Smolko and O. Amin, 2005. Effects of starvation on oxygen consumption, ammonia excretion and biochemical composition of the hepatopancreas on adult males of the False Southern King crab *Paralomis granulosa* (Crustacea, Decapoda). Comp. Biochem. Physiol. B. Biochem. Mol. Biol., 140: 411-416.

Dall, W., 1981. Osmoregulatory ability and juvenile habitat preference in some penaid prawns. J. Exp. Mar. Biol. Ecol., 54: 55-64.

Davis, C.W. and I.R. Hagadorn, 1982. Neuroendocrine control of Na+ balance in the fiddler crab *Uca pugilator*. Am. J. Physiol., 242: 505-513.

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

DuBois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.

Eckhardt, E., C. Pierrot, P. Thuet, F. Van Herp, M. Charmantier-Daures, J.P. Trilles, F. Charmantier, 1995. Stimulation of osmoregulating processes in the perfused gill of the crab *Pachygrapsus marmoratus* (Crustacea, Decapoda) by a sinus gland peptide. Gen. Comp. Endocrinol., 99: 169-177.

Folch, J., M. Lees and G.H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.

Highnam, H.C. and L. Hill, 1979. The Comparative Endocrinology of the Invertebrates. Edward Arnold Publishers, London, pp: 209.

Koshio, S., S. Teshima and A. Kanazawa, 1992. Effects of unilateral eyestalk ablation and feeding frequencies on growth, survival and body compositions of juvenile fresh water prawn *Macrobrachium rosenbergii*. Nip. Sui. Gak., 58: 1419-1425.

Lin, J., D. Zhang and R.L.R. Crewell, 2001. Aquaculture. Lake Buera Vista, Florida, USA., pp: 378.

Madyasthan, M.N. and P.V. Rengnekhar, 1976. Metabolic effects of eyestalk removal in crab *Varana litterata*. Hydrobiologia, 48: 25-25.

McNamara, J.C., L.C. Salomao and E.A. Ribeiro, 1990. The effect of eyestalk ablation on haemolymph osmotic and ionic concentrations during acute salinity exposure in the freshwater shrimp *Macrobrachium olfersii* (Wiegmann) (Crustacea, Decapoda). Hydrobiologia, 199: 193-199.

Morris, S., 2001. Neuroendocrine regulation of osmoregulation and the evolution of air-breathing in decapod crustaceans. J. Exp. Biol., 204: 979-989.

Mourente, G. and A. Rodriguez, 1991. Variation in the lipid content of wild-caught females of the marine shrimp *Penaeus kerathllrus* during sexual maturation. Mar. Biol., 110: 21-28.

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

Murugesan, R., P. Soundarapandian and K. Manivannan, 2008. Effect of unilateral eyestalk ablation on the biochemical changes of edible portunid crab *Charybdis lucifera* (Fabricius). J. Fish. Aquatic Sci., 3: 82-86.

Nan, F.H., M.H. Hong, C.L. Lu and S.S. Sheen, 2004. The effect of unilateral and bilateral eyestalk ablation on haemolymph osmotic and ionic regulation and gill Na+, K+ATPASE activity in *Marsupenaeus japonicus* (Bate, 1888) (Decapoda, Natantia). Crustaceana, 77: 385-395.

Oliveira, G.T., F.A. Fernandes, G. Bond-Buckup, A.A. Bueno and R.S.M. Da Silva, 2003. Circadian and seasonal variations in the metabolism of carbohydrates in *Aegla ligulata* (Crustacea: Anomura: Aeglidae). Mem. Museum Victoria, 60: 59-62.

Oliveira, G.T., I.C. Rossi, L.C. Kucharski and R.S.M. Da Silva, 2004. Hepatopancreas gluconeogenesis and glycogen content during fasting in crabs previously maintained on a high-protein or carbohydrate-rich diet. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 137: 383-390.

Quinn, I.E. and C.E. Lane, 1966. Ionic regulation and Na^+-K^+ stimulated ATPase activity in the land crab. *Cardiosoma quanhnmi*. Comp. Biochem. Physiol., 19: 533-543.

Ravichandran, S. and T. Kannupandi, 2004. Biochemical changes in decomposing leaves and crabs of *Pichavaram mangroves*. Biochem. Cell. Arch., 24: 79-84.

Ravichandran, S., G. Rameshkumar, S. Velankanni and T.T. Ajithkumar, 2009. Variation in lipid concentration of the crab *Portunus sanguinolentus* at different developmental stages. Middle-East. J. Sci. Res., 4: 175-179.

Raymont, J.E.G., A. Austine and E. Lingford, 1964. Biochemical studies on marine zooplankton. I. The biochemical composition of *Neonysis interger*. J. Cons. Int. Explor. Mer., 28: 354-363.

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

Rudy, P.P., 1966. Dodium balance in *Pachygrapsus carassipes*. Comp. Biochem. Physiol., 18: 881-907.

Sainath, S.B. and P.S. Reddy, 2010. Melatonergic regulation of hemolymph sugar levels in the freshwater edible crab, *Oziotelphusa senex senex*. J. Exp. Zool. A Ecol. Gene. Physiol., 313: 201-208.

Samuel, J.N., N. Thirunavukkarasu, P. Soundarapandian, A. Shanmugam and Kannupandi, 2004. Fishery potential of commercially important portunid crabs along parangipettai coast. Proceedings of International Conference and Exposition on Marine Living Resources of India for Food and Medicine, Feb. 27-29, Aquaculture Foundation of India, Chennai, pp: 165-173.

Schoenfeld, R.S. and C.J. Lewellen, 1964. A colorimetric method for determination of serum chloride. Clin. Chem., 10: 533-539.

Serrano, L., G. Balanvillain, D. Soyez, G. Charmantier, E. Grousset, F. Aujoulat and C. Spanings-Pierrot, 2003. Putative involvement of crustacean hyperglycemic hormone isoforms in the neuroendocrine mediation of osmoregulation in the crayfish *Astacus leptodactylus*. J. Exp. Biol., 206: 979-988.

Shah, A.S.I., 1995. Comparison of growth and conversion efficiency of *Macrobrachium malcolmsonii* (H. Milne Edwards) juveniles fed with different commercial and formulated feeds. M.Sc. Thesis, Annamalai University, India.

Sheen, S.S. and L.R.D. D'Abramo, 1991. Response of juvenile freshwater prawn, *Macrobrachium rosenbergii*, to different levels of cod liver/corn oil mixture in a semi-purified diet. Aquaculture, 93: 121-134.

Soundarapandian, P. and G. Ananthan, 2008. Effect of unilateral eyestalk ablation on the biochemical composition of commercially important Juveniles of *Macrobrachium malcolmsonii* (H. Milne Edwards). Int. J. Zool. Res., 4: 106-112.

Soundarapandian, P. and R.K. Singh, 2008. Biochemical composition of the eggs of commercially important crab *Portunus pelagicus* (Linnaeus). Int. J. Zool. Res., 4: 53-58.

Spanings-Pierrot, C., D. Soyez, V.F. Herp, M. Gompel, G. Skaret, E. Grousset, G. Charmantier, 2000. Involvement of crustacaen hyperglycemic neurohormone in the control of gill ion transport in the crab *Pachygrapsus marmoratus*. Gen. Comp. Endocrinol., 119: 340-350.

Surendranath, P., K. Ramanaiah and K.V.R. Rao, 1992. Effect of eyestalk extract and Kelthane on penaeid prawn *Metapenaeus monoceros* (Fabricus). Indian J. Mar. Sci., 30: 676-679.

Tierney, A.J., T. Kim and R. Abrams, 2003. Dopamine in crayfish and other crustaceans: Distribution in the central nervous system and physiological functions. Microsc. Res. Tech., 60: 325-335.

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.

Vernberg, F.J. and W.B. Vernberg, 1974. Pollution and Physiology of Marine Organisms. Academic Press, New York, pp: 492.

Vinagre, A.S. and R.S.M. Da Silva, 1992. Effects of starvation on the carbohydrate and lipid metabolism in crabs previously maintained on a high protein or carbohydrate-rich diet. Comp. Biochem. Physiol. A Physiol., 102: 579-583.

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

Zafar, M., M.Z.H. Siddiqui and M.A. Hoque, 2004. Biochemical composition in *Scylla serrata* (Forskal) of Chakaria Sundarban area, Bangladesh. Pak. J. Biol. Sci., 7: 2182-2186.

Zaib-Un-Nisa and A. Muzammil, 2001. Natural and induced spawning of Pakistan Penaeid shrimps in the laboratory. Aquaculture, 33: 125-130.

Zanders, I.P., 1980. Regulation of blood ions in *Carcinus maenas* (L.). Comp. Biochem. Physiol. B Physiol., 65: 97-108.