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Haemolymph protein concentration in freshwater crab, *Sartoriana spinigera* (Wood Mason, 1871)

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Abstract : The freshwater crabs (Decapoda-Crustacea) *Sartoriana spinigera* are abundantly found in large numbers in shoreline areas of the ponds, lakes, wetlands and paddy fields of Jharkhand.

Very little is known about the basic biology of this organism. Basic information of its life cycle and physiological parameters are completely lacking. To evaluate the health and physiology of this wild population of crab, biochemical parameters of its haemolymph were determined from collection of adult (male & female). Crab's haemolymph parameters fluctuate according to their external environment, developmental stage and reproductive cycle. Variation in the biochemical composition of total proteins, has been studied. It has been observed that all these parameters are functions of sex.

Total plasma protein concentration in different weight groups of adult male (18.68-90.56g) and adult female (24-57.3g) was 5.52+1.76 g/dl and 3.71+1.22g/dl respectively. The differences between these means were statistically significant ($p<0.001$). The plasma protein concentration in male showed a significant positive correlation with body weight ($r=0.969; p<0.001$). But in female such relationship was not found.

In the present paper the reason and significance for all these observations were discussed in details.

Keywords : Haemolymph, plasma protein, *S. spinigera*

INTRODUCTION

Proteins are the most abundant compounds in serum, because they are the basic component of enzymes, many hormones, antibodies and clotting agents. It plays a major role in maintaining the delicate acid base balance.

The serum proteins serve as a reserve source of energy for the tissue and the muscle, but environmental as well as sexual changes often disturb concentration of haemolymph protein and also disturb the acid base balance. Edward and Marilyn (1963) also reported the variability in the concentration of protein in Callinectes sapidus, the common Atlantic coast blue crab, and published its data on the range and the mean values for the protein concentration in the adult of this species. Considerable amount of informations are available for many Decapods, particularly European species. Edward and Marilyn (1963) determined that the haemolymph protein concentration in Callinectes sapidus and accumulated two facts (i) individual variation of haemolymph protein concentration showing ten - fold range and (ii) males crabs showed a distinctly lower mean value than females for protein concentration.

Edward and Marilyn (1963) also observed that one half of the serum protein was haemocyanin. In the present investigation plasma was assayed to provide an indication of protein component which varied sufficiently in their concentration to produce the wide range in individuals total protein concentration.

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MATERIALS AND METHODS

The freshwater crab Sartoriana spinigera was collected from the local market and brought to the laboratory of Ranchi College, Ranchi and was kept in large sized aquarium having little water and sand. The crabs were acclimated to laboratory conditions for one week.

The water was changed alternatively. Crabs were not fed during the experimental period. Subsequently adult males and females were separated by observing the abdomen and considering the body weight. Crabs having body weight less than 20g were selected as juvenile and more than that were selected for adult group.

Before taking haemolymph samples experimental crabs were weighed. Haemolymph sample were taken withdrawing 0.5 to 1 ml haemolymph from each crab using hypodermic needle, inserted through the arthrodial membrane at the base of chelate or 4th or 5th walking legs. These samples were used for the determination of total protein. Haemolymph were centrifuged for five minutes at 3000 rpm and supernatant was taken for estimation. Total protein was determined by Lowrey et.al (1951) method using bovine serum albumin as a standard. The intensity of blue color developed was proportional to the concentration of plasma protein which was read at 546nm in semi - autoanalyser (Erba Mannheim - CHEM 5 Plus V2).

RESULTS

Total plasma protein concentration in different weight groups of adult males (18.68-90.56g) and adult females (24-57.3g) are shown in Table-I and Table-II respectively. Average total plasma protein concentration in adult male was 5.52+1.76 g/dl, whereas average total plasma protein concentration in female was only 3.71+ 1.22 g/dl. These means were analyzed statistically using Student's t - test and it showed that the different between these two means were highly significant ($p<0.001$).

When the data for plasma protein concentration in adult male crabs were plotted against body weight, they depicted a straight line with a slope value ($p=0.062$) and intercept ($a=2.060$). The plasma protein concentration showed a positive correlation with body weight ($r=0.969$; $p<0.001$) (Fig. - I). Fig.- II showed graph plotted between body weight and protein concentration in adult female crabs, which showed slight negative correlation ($r=-0.192$).

Table-I : Estimated value of total plasma protein concentration (g/dl) in different weight groups of adult male crabs, S. spinigera

Sl. No.	Body weight of adult male crabs	Total plasma protein conc. (g/dl)
1	90.56	8.39
2	88.2	7.12
3	85.49	7.05
4	82.78	6.93
5	80.07	6.85
6	77.36	6.8
7	71.94	6.65
8	66.52	6.49
9	58.72	6.16
10	49.3	5.46
11	38.56	5
12	31.68	4.6
13	27.72	3.96
14	24.08	3.78
15	21.3	2.68
16	20.44	2.55
17	18.68	3.24
Average		5.512353
S.D		+ 1.76469
r		0.968587

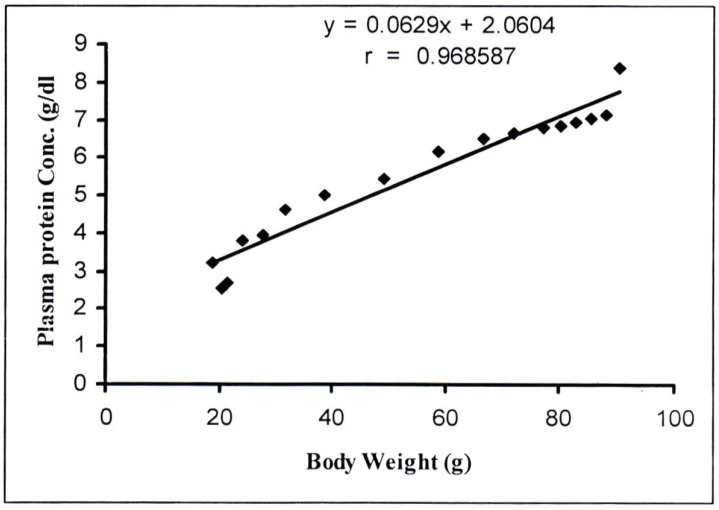

Fig:-1 Graph between Body weights (g) of adult male Vs plama protein concentration(g/dl) in S. spinigera

Table-II : Estimated Value for total plasma protein concentration (g/dl) in different weight groups of adults female crabs, S. spinigera

Sl. No.	Body weight of adult female Crab (g)	Total plasma protein conc. (g/dl)
1	57.3	3.27
2	57.16	3.74
3	50.68	2.86
4	44.44	4.63
5	42.22	2.86
6	41.1	5.24
7	39.62	3.67
8	39.04	3.51
9	36.66	3.2
10	35	3.98
11	34.92	4.68
12	33.26	3.01
13	24	3.96
Av.		3.739231
SD		+ 0.746307
r		-019296

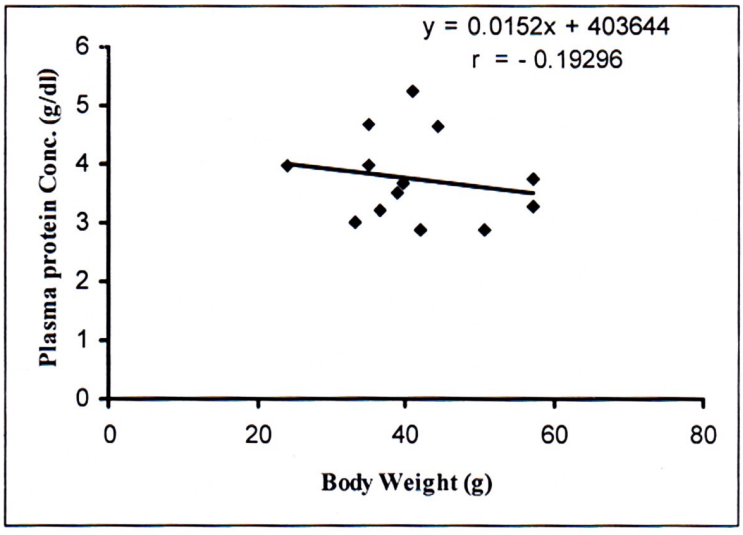

Fig.2 Graph between Body weight of adult female (g) Vs plasma protein concentration (g/dl) in S.spinigera

DISCUSSION

Present investigation showed that freshwater crab's haemolymph protein concentration is a function of sex and size, physiological condition as well as reproductive cycle of the crab. Adult male contained significantly higher concentration of the total plasma protein (5.52 g/dl) than adult female (3.6 g/dl + 1.22), which signified more growth in males than females. In adult males the effect of size is expressed as a power function of body weight. There is a positive correlation between the mass of crab and the concentration of plasma protein. Sinha and Ahmed (1978) also reported variation in the biochemical composition of tissue protein in the crab, Sesarma' boulengeri. But in contrast to present finding they reported negative correlation between body weight and tissue protein concentration i.e lower the body weight, higher the plasma protein concentration. It signified that young male crab had higher metabolic rate as well as highter growth rate. Stephen et al (2002) studied the horse shoe crab, Limulus polyphemus haemolymph biochemical parameters and reported that the biochemical values fluctuate their osmolality and other haemolymph parameters according to their external environment, developmental stage and reproductive cycle. According to them total plasma protein concentration in Limulus polyphemus was 8.15 g/dl. This value is slightly higher than the present study i.e. 5.51 g/dl. Total haemolymph protein concentration in Decapods were known to vary widely both inter and intra specifically (Depledge & Bjerragaard, 1989). Bjerregaard and Vislie (1986) measured the value of protein in Carcinus maenas as 5.7 g/dl, which is very close to our estimated value in adult male crab. Horn and Kerr (1963) also studied the variability in the haemolymph proteins in Callinectes sapidus Rathbun, blue crab and reported that males showed a distinctly lower mean value than females for protein concentration which contradicts the present findings. It may be due to the difference in reproductive stage of crabs, because samples were collected from the female in the month of July and August, 2007, when the females were in post - oviposition stage i.e. ovary were in resting or spent phase. Several investigators (Gray and New Combe, 1938 : Tyler and Cargo, 1963) also led us

to the conclusion that there are wide size variations in the adults of both sexes. Edward and Marylin (1963) reported that in adult blue crab, Callinectes sapidus male serum protein concentration ranged between 8.8-132 mg/ml, (Av 52.37 mg/ml or 5.37 g/dl) which is quite similar to our observation in adult male crab. But in case of female blue crab they reported that it ranged between (15.2-119.2 mg/ml, (Av 62.70 mg/ml or 6.7 g/dl), which is quite higher from present finding. This difference may be due to change in sampling or experimental time. The higher concentration of female protein may be definitely due to presence of female specific proteins during vitellogenesis and oocyte development.

Another important observation was that in adult females the correlation between body weight and plasma protein concentration was slightly negative (r = -0.1917), this fluctuation was only due to the presence of different concentration of female specific protein (FSP) during the different stages of oocyte development. Lee (1990) also reported that Vg (vitellogenin) was one of the two lipoproteins present in female crustacean haemolymph. Its concentration in- ceased during vitellogenesis. Byard and Atken (1984) als reported that FSP in the haemolymph were always higher prior to the maximum accumulation of yolk in the oocytes, but they dropped off markedly prior to oviposition. The FSP is the part of protein, which is externally synthesized and is found at the highest level in the haemolymph during the period, when oocytes are accumulating the maximum amount of yolk.

Bakker et al (1977) also reported that in insect also Vg is produced by an extra ovarian tissue and released into the haemolymph and subsequently taken up by the developing oocytes. Shafir et al (1992) in Penaeus senaisuleatus (de Haan) also reported similar findings that concentration of total protein heamolymph increased with progress of ovarian development shown by the increase in average oocyte diameter (AOD). The reported the highest average values of protein (2.47 g/dl) and which were found in females with an AOD ranging between 250 and 300 m. Lee and Puppione (1988) also mentioned similar relationship in protein increase in the haemolymph and oocyte development in Callinectes sapidus.

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