

## STUDIES ON HAEMOLYMPH PROTEIN AND COPPER CONSTITUENTS IN GHOST CRAB *OCYPODE CORDIMANA*

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### ABSTRACT

The haemolymph protein and copper (free and bound) concentration of a ghost crab *Ocypode cordimana* has been studied in two size groups of males and females separately. These haemolymph constituents have been analysed in relation to the sex differences in male and female in order to understand their concentrations and the ratio between protein and copper, and also its relationship between copper free and bound proteins.

### INTRODUCTION

THE GHOST CRAB *Ocypode cordimana* belongs to the family Ocypodidae, which inhabit the tidal zone. Ocypoda is a major group which includes many nocturnal crabs, occupying different habitats of intertidal zone. So far nineteen species of ocypodes have been reported of which only five have been reported in Indian shores (Alcock, 1900; Chhappgar, 1957). Among them *O. cordimana* is supralittoral and is more terrestrial than other species (Fellows, 1973). Sometimes the burrows of *O. cordimana* are found 200 yards away from the wave wash zone (Rao, 1968). This crab differs from all other species of ocypodes in the absence of eyestylet and stridulating ridges on the inner surface of the chela propodus. These crabs are slow moving and docile in contrast to other ghost crabs. Paulraj (1980) has studied the morphology, morphometry, sexual maturity, burrow pattern, autotomy and desiccation tolerance of *O. cordimana*. The body size is much reduced and the onset of sexual maturity is earlier in this ghost crab. According to

Paulraj (1980) *O. cordimana* constructs predominantly 'y' shaped burrows. It is also primarily adopted to the stress and desiccation. The present investigation aims at finding various concentrations of haemolymph protein, copper, their ratio and the relationship existing between the copper free and bound proteins of *O. cordimana* in two groups of males and females.

### MATERIALS AND METHODS

*Ocypode cordimana* was collected by hand picking from the wave wash zone of Madras Beach. Their distribution is very sparse as evidenced by the number of burrows 10-70 for 10 m<sup>2</sup>. The animals were divided into two groups based on their carapace width (Group I consists of animals having less than 21.5 mm Carapace width and Group II between 21.5 mm and 30 mm carapace width). Before taking measurements, animals were immobilized by placing them in the ice chamber of a refrigerator for 15 to 20 minutes. A pair of finely pointed divider and a millimeter scale were used for

taking the measurements. The entire investigation was carried out on the blood of *O. cordimana* and it satisfies the requirement. Blood was directly drawn into a fine calibrated 0.1 ml micropipette by cutting the tip of the leg and was immediately transferred to the tubes containing the required reagents for various biochemical analyses. Care was taken to see that seepage was minimal.

The haemolymph protein concentration of *O. cordimana* was determined by following the Biuret method of Gornall *et al.* (1949), because the consistency of this method has been

established by Subhashini (1981) and Subhashini and Ravindranath (1980). For the determination of copper, 2, 2' biquinoline spectrophotometric method was used, which is very reliable, simple, stable and reproducible (Arumugam, 1981; Arumugam and Ravindranath, 1980, 1981).

#### RESULTS

The haemolymph constituents like protein and copper in two size groups of males and females of *O. cordimana* studied, the test of significance was worked out and the results presented in Table 1.

TABLE 1. *Haemolymph constituents of Ocy de cordimana*

Haemolymph constituents	Sex	Groups		Test of Significance	
		I	II		
Protein concentration (g%)	Male	12.499 ± 2.119 (5) NS	14.08 ± 1.846 (6) NS	NS	
	Female	13.175 ± 2.125 (2)	14.244 ± 1.042 (6)	NS	
Copper concentration (µg/ml)	Male	134.92 ± 20.283 (5) (P < 0.01)	142.965 ± 25.979 (6) (P < 0.001)	NS	
	Female	112.42 ± 13.250 (2)	119.814 ± 21.415 (6)	NS	
Copper Protein ratio	Male	0.0902 ± 0.003 (5) NS	0.0983 ± 0.009 (6) NS	NS	
	Female	0.0858 ± 0.003 (2)	0.0822 ± 0.009 (5)	NS	
Copper Free Protein (gm%)	Male	6.826 ± 1.157 (5)	7.064 ± 0.737 (8)	NS	
	Female	7.575 ± 1.453 (5)	7.705 ± 0.255 (5)	NS	
Copper Bound Protein (gm%)	Male	5.673 ± 1.107 (5) NS	7.015 ± 1.382 (6) NS	NS	
	Female	5.601 ± 0.672 (2)	5.978 ± 1.074 (5)	NS	

Values are expressed as mean ± S.E.; Sample size is given in parentheses; Statistically significant levels are given brackets; NS - not Significant.

### Protein concentration

The protein concentration varied much between sexes. But it is not statistically significant between two groups. In males it ranged from 12.499 gm% in group I to 14.08 gm% in group II. In females it ranged from 13.175 gm% in group I to 14.244 gm% in group II. It is also statistically not significant.

### Copper concentration

The copper concentration varied between sexes in stages I and II. The copper concentration ranged from 134.92 µg/ml to 142.965 µg/ml between groups I and II respectively in males. Similarly it ranged from 112.42 µg/ml to 119.814 µg/ml between groups I and II in female. In general the copper concentration value was higher in males than in females. There was an increase in the copper concentration from group I to II.

### Copper protein ratio

The copper protein ratio analyses in the haemolymph of *O. cordimana* revealed much less difference among males and females and also between the two groups. The copper protein ratio helped in finding the quantities of copper bound and copper free proteins.

### Copper free protein

The copper free protein level differed in groups I and II among males and females. In group I of *O. cordimana*, it was more than in group II. The copper free protein was found to be higher in females of group I among all.

### Copper bound protein

The copper bound protein concentration in the haemolymph of *O. cordimana* was higher in males of group II than the rest. There was very little difference between males and females of group I. But there was considerable difference

between males and females of group II in copper bound protein level.

### DISCUSSION

As reported by Subhashini and Ravindranath (1980), in general haemolymph water content in crabs ranges from 90 to 95% and the rest is dry weight with major share of protein. The protein concentration in *O. cordimana* ranges from 12.499 gm% to 14.244 gm%. In brachyuran decapods the haemolymph protein concentration rarely exceeds 10 gm%. In *Macrobrachium holstatus*, it ranges from 1.52 to 9.42 gm% (Uglow, 1969). In *Callinectes sapidus*, it ranges from 1.46 to 10.0 gm% (Horn and Kerr, 1963; Lynch and Webb, 1973). In *Scylla serrata* it ranges from 2.72 to 13.0 gm% (Subhashini and Ravindranath, 1980; Arumugam and Ravindranath, 1980). In *Ocypode platytarsis* the protein concentration ranges from 7.61 to 1.23 gm% (Anvar Batcha, 1991). From the above reports, it may be noted that the range of haemolymph protein concentration observed in *Ocypode cordimana* is similar, as observed by earlier workers in other crustaceans mentioned above.

The haemolymph concentration also differed between sexes in *O. cordimana* in group I and II. Higher values are recorded in females than in males in both groups. Balazs *et al.* (1974) have reported similar results in *Macrobrachium rosenbergii* and *Penaeus marginatus*.

The present study also shows that there is a close relationship between haemolymph protein and copper concentration. It is in close agreement with the findings of Horn and Kerr (1969) and Colvocoresses and Lynch (1975) in the blue crab *Callinectes sapidus* and that of Arumugam (1981) in the mud crab *Scylla serrata*. The range of copper concentration reported for *O. cordimana* in the present

investigation is also comparable to *crangon vulgaris* (Djanmah and Grove, 1970), *Scylla serrata* (Arumugam and Ravindranath, 1980) and *Ocypode platytarsis* (Anvar Batcha, 1991).

The copper protein ratio of *O. cordimana* ranges from 0.0822 to 0.0983 which is comparable to that of *Eriocheir sinensis* (Gilles, 1977). Only puse haemocyanin is known to have the higher copper protein ratio of 0.2. But the low copper protein ratio reported for *O. cordimana* suggests that copper free proteins may be present in this species. From the ratio it is obvious that *O. cordimana* may contain less of copper bearing proteins. Paulraj (1980)

has reported that *O. platytarsis* is having considerably more copper bearing proteins and so it is agile in nature, as it is littoral in habitat. Whereas in the present study in *O. cordimana* comparatively lesser copper protein ratio is reported which shows that this species may not require more copper bearing proteins, because it is more or less terrestrial in habitat and docile in action. Incidentally among both the groups of *O. cordimana* the copper free protein shows higher value in females when compared to males. On the other hand the copper bound protein is slightly higher in males when compared to females in groups I and II of *O. cordimana*.

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