

**IMPACT OF SEX, SEXUAL MATURITY AND HABITAT
ON THE HAEMOLYMPH PROTEIN AND COPPER OF
THE GHOST CRAB *OCYPODE PLATYTARSIS* (MILNE EDWARDS)**

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ABSTRACT

The haemolymph protein and copper concentration of *Ocypode platytarsis* has been studied in three size groups of males and females separately. These haemolymph constituents have been analysed in greater detail in relation to the sex and sex differences of *Ocypode platytarsis* with a view to find out the impact of sexual maturity and habitat of males and females on the haemolymph protein and copper constituents.

INTRODUCTION

THE MEMBERS of the family Ocypodidae inhabit the tidal zone. One of the major genera of the family is *Ocypode* which are commonly called Ghost crabs. These are nocturnal crabs found occupying different habitats of the intertidal zone. Nineteen species of *Ocypode* crabs have been reported so far, of which only five have been reported in Indian shores (Alcock, 1900 ; Chhapgar, 1957). Among the five, *O. platytarsis* is littoral in habitat (Rajabai Naidu, 1951 ; Takahasi, 1935). Paulraj (1980) has distinguished the stages of sexual maturity as immature, maturing and matured. Male members of *O. platytarsis* mature when the carapace width is between 20 and 30 mm and female members mature when the carapace width is between 30 and 35 mm. The present investigation aims at finding the impact of sex, sexual maturity and habitat on the haemolymph protein and copper concentration. Attempt has also been made to study the copper and protein relationships.

MATERIALS AND METHODS

For the purpose of present investigation *O. platytarsis* was collected from the wave

wash zone of Madras Beach. The animals were collected at night using dragnet. Measurements of Carapace width and length of the crabs were taken alongwith weight following the procedures of Sandan (1937) and Raghu Prasad and Tampi (1954). The method used to distinguish the stages of maturity of sex was by the criteria suggested by Paulraj (1980). The animals were divided into 3 groups on the basis of their carapace width (Group I consists of animals having less than 21.5 mm carapace width ; Group II between 21.5 and 30.0 mm and Group III more than 30.0 mm carapace width). Before taking measurements, the animals were immobilized by placing them in the ice chamber of the refrigerator for about 3 minutes. A pair of finely pointed divider and a millimeter scale were used to take the carapace width. The weight of the crabs were taken using the Stanton DO3T mono balance.

Blood sample

The entire investigation was carried out on the blood of *O. platytarsis*. Hence it becomes necessary that the blood available from the crab must be of significant quantity. In this regard *O. platytarsis* satisfies the requirements.

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 analysis. Care was taken to see that seepage was minimal.

Estimation of protein

The haemolymph protein concentration of the crabs was determined following Biuret method of Gornall *et al.* (1949). This method was selected, because the consistency of this method has been established by Subashini (1980) and Subhashini and Ravindranath (1980, 1981) and found this method superior for crustacean blood proteins over Folin-ciocalteu and microkjeldahl methods, due to its greater consistency in performance, simplicity and stability of colour and least susceptibility for interfering substances.

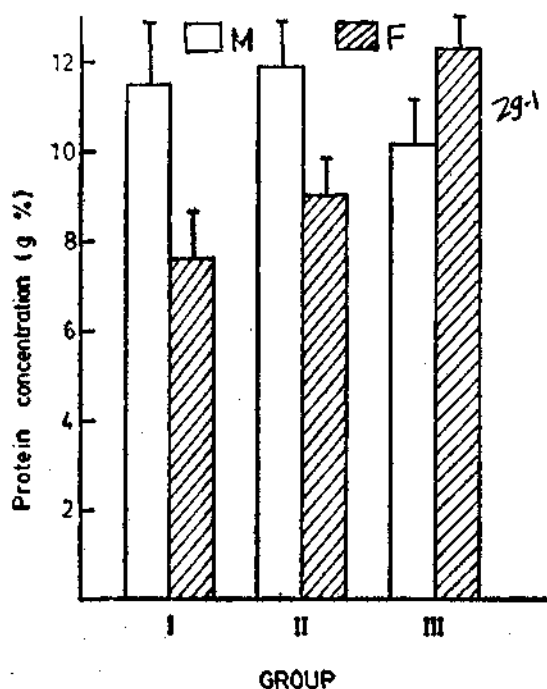


Fig. 1. Protein concentration (g %) at different groups.

Determination of copper

In the present study 2, 2', biquinoline method is used which is the most common spectrophotometric method, because it is very reliable, simple, stable and reproducible (Arumugam and Ravindranath, 1980, 1981).

RESULTS

The haemolymph constituents of *O. platytarsis* are studied in three size groups of males and females separately. The haemolymph constituents analysed include protein and copper.

Protein

The protein concentration differs between sexes (Fig. 1). The sex difference, though obvious in groups I and II, it is statistically significant in group II. In males, in general, the protein level ranges from 10.16 to 11.91 gm %. In females it ranges from 7.61 to 12.3 gm %. There is no difference in the protein concentration of males in relation to sexual maturity. On the other hand, the protein level increases steadily during the course of sexual maturity in females. The differences between groups II and III and I and III are significant.

Copper

The copper concentration differs between sexes in stages I, but such differences is not obvious in later stages of maturity (Fig. 2). Among the male members of the groups, the copper concentration ranges from 108.2 to 144.02 $\mu\text{g/ml}$. The difference between groups are not statistically significant. Among female members of the groups, the copper level increases from 92.03 to 128.37 $\mu\text{g/ml}$. The difference between groups I and III is statistically significant.

Copper and protein relationship

Results of analysis of copper and protein concentrations reveal that there is a significant positive correlation between protein and copper concentrations (Fig. 3). The slope of regression

is statistically significant. The regression analysis confirms the close association between the two constituents. Based on the data, copper/protein ratio was calculated. Interestingly the ratio did not differ between sexes and stages of sexual maturity between males and females. The copper protein ratio has enabled calculating the quantities of copper bound and copper free proteins.

of sexual maturity, the copper free proteins differ between stages II and III in both sexes. In males, there is a fall in copper free protein level in stage III. In females the reverse is true.

DISCUSSION

It is known that in general haemolymph water content in crabs ranges from 90 to 95%

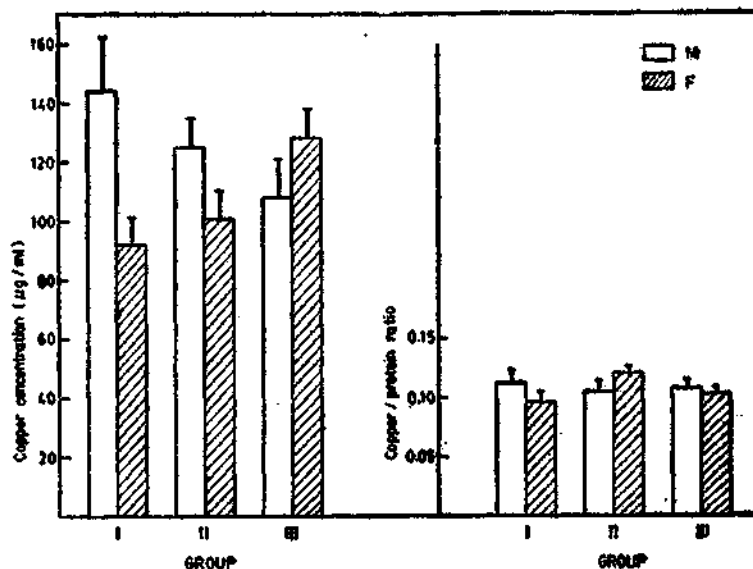


Fig. 2. Copper concentration ($\mu\text{g/ml}$) and copper-protein ratio in different groups.

Copper bound proteins

The copper bound proteins do not vary much in relation to sexual maturity in males (Fig. 4). On the other hand, significant difference is observed between stages I and II and I and III in females. The copper bound protein differs between sexes in group I, but such sex differences is not observed at later stages.

Copper free proteins

Copper free proteins (Fig. 4) differ between sexes in group II, in that they are higher in males than in females. Considering the stages

and the rest is dry weight with major share of protein (Subhashini and Ravindranath, 1982). The protein concentration in the haemolymph ranges from 7.61 to 12.3 gm % in *O. platytarsis*. Usually in brachyuran decapods haemolymph protein concentration rarely exceeded 10 gm %. In *Macropus holstatus*, it ranged from 1.52 to 9.42 gm % (Uglow, 1969); in *Callinectes sapidus*, it ranged from 1.46 to 10.0 gm % (Horn and Kerr, 1963; Lynch and Webb, 1973; Pauley *et al.*, 1975); in *Scylla serrata* it ranged from 2.72 to 13.0 gm % (Subhashini and Ravindranath, 1980; Arumugam and Ravindranath, 1980). It may be noted from the above given reports that the range of

haemolymph protein values observed for *O. platytarsis* is not uncommon in previous literature. But Ramalingam *et al.* (1981) have found that the haemolymph protein concentra-

tion factor 6.25 and the conversion factor is applicable only to pure proteins and not to protein bound with lipids and polysaccharides (Bailey, 1967). It is known that a major fraction of haemolymph protein of the crabs is bound with lipids and polysaccharides (Paulpandian and Kannupandi, 1975).

The protein concentration in the haemolymph also differed between sexes in *O. platytarsis*. In stage II sexual maturity in males showed higher values than females. Similar results have been reported in *Penaeus marginatus* and *Macrobrachium rosenbergii* by Blaze *et al.* (1974).

The present investigation reveals that there is a close positive correlation between haemolymph copper and protein concentration, a finding in close agreement with the reports of Horn and Kerr (1963) and Colvocoresses and Lynch (1975) for the Blue crab *Callinectes sapidus* and that of Arumugam (1981) for the Mud crab *Scylla serrata*. The range of copper concentration reported for *O. platytarsis* is comparable to *Palaemon squilla*, *Crangon vulgaris* (Djangmah and Grove, 1970) *Palinurus interruptus* (Johnston and Barber, 1969) and *Scylla serrata* (Arumugam and Ravindranath, 1980).

The copper protein ratio of *O. platytarsis* ranged from 0.094 to 0.121 which is comparable to that of *Callinectes sapidus* (Horn and Kerr, 1969). Only pure haemocyanin is known to have the copper protein ratio of 0.2. But the low copper protein ratio reported for *O. platytarsis* suggests that copper free proteins may be present in this species. From the ratio it is clear that *O. platytarsis* is having considerably more copper bearing proteins and so it is agile as reported by Paulraj (1980). Since the major proportions of copper bearing proteins are oxygen carriers greater demand is there in

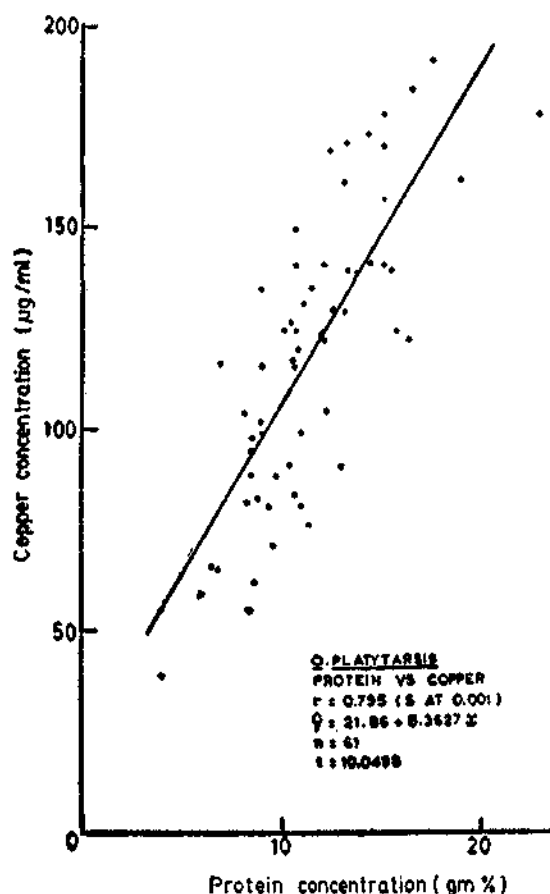


Fig. 3. Copper - Protein relationship in *O. platytarsis*.

tion is *O. platytarsis* ranged from 3 to 6 gm %. Such low values reported for *O. platytarsis* may be due to the methodology adopted by Ramalingam *et al.* (1981). It was shown that haemolymph protein value in general is always lower with Micro-Kjeldahl method, when compared with Biuret and Folin-ciocalteu methods (Subhashini and Ravindranath, 1980), because the protein value calculated after multiplying the nitrogen value with a conver-

the littoral and agile *O. platytarsis* which requires more of copper bound protein. It is interesting to note that the copper bearing

one may note that there is an increase in the level of copper bearing proteins in the sexual maturity in females. Probably these proteins

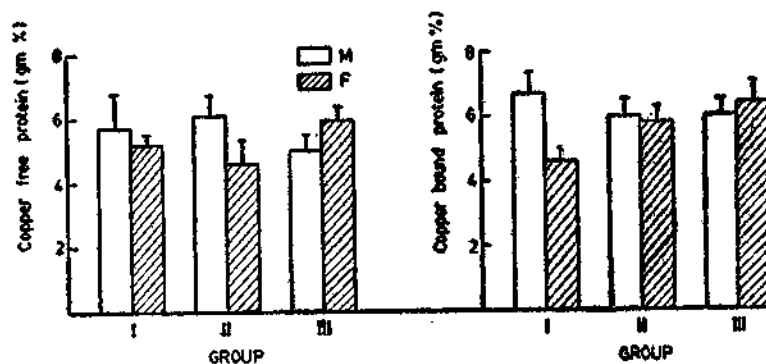


Fig. 4. Copper-free and copper-bound protein (g %) in different groups.

proteins do not change much in relation to sexual maturity in *O. platytarsis*. However

are necessary for the reproductive activities of the females and also for egg production.

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