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PROLINE ACCUMULATION OF *SESARMA BROCKII* DE MAN LARVAE IN RESPONSE TO THE LARVAL DEVELOPMENT AND SALINITY STRESS

ABSTRACT

The 5 larval stages (4 zoea & 1 megalopa) were exposed to different salinities ranging from 5 to 40 ppt with 5 ppt. increment and the proline content was estimated in each stage. The mean proline content increased with not only increased salinity but also with the stage of larval development. The result was subjected to 't' test and 'ANOVA' and they were found to be significant at 5% level.

AMINO ACIDS are known to play an important role in the life-cycle of crustaceans (Florkin and Scheer, 1970) and more specifically during osmotic stress (Virkar and Webb, 1970). Further, the amino acids are essential intermediates in protein synthesis. Among the free amino acids (FAA), proline (Pyrrolidine -2- Carboxylic acid) is well known for its phenomenal accumulation in biological system under saline stress (Kathiresan, 1983). However, no such serious attempt has been made to investigate proline accumulation in estuarine and mangrove animals which are subjected to saline stress. This prompted the study of proline accumulation during larval development of *S. brockii* in response to its development and to changes in environmental salinity.

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MATERIAL AND METHODS

Ovigerous females of *S. brockii* were collected from Pichavaram mangroves and maintained in the laboratory at a salinity of 25±1 ppt, a temperature of 28±1°C and a photoperiod of 12h L: 12h D. After hatching the larvae were utilized for the analysis proline content.

The proline content of the samples was estimated using the method of Bates *et al.* (1973) with modification by Kathiresan (1983). Five mg. of newly hatched larvae were rinsed with distilled water and homogenized with 2 ml of 2% sulphosalicylic acid using pestle and mortar. The homogenate was centrifuged for 5 minutes at 4000 g to get a clear supernatant which was the proline source. 2 ml of this sample was mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin reagent. The tubes containing the mixture were incubated in a boiling water bath for 1 hour at 100°C and then cooled down by placing the tubes in an ice bath. Four ml of toluene was added to the mixture which was then shaken. The mixture was allowed to separate into two layers for 10-15 minutes. The upper layer was eluted using a pasteur pipette. The color intensity was read at 520 nm with a CECIL-303-Spectrophotometer. The same procedure was

adopted for the standard and blank also. The different stages were exposed to different salinities for 24 hours and proline was analysed. The proline content of the sample was calculated using the formula.

$$\text{Proline content} = \frac{34.48 \times \text{Volume of total extraction}}{\text{Fresh wt. of larvae} \times \text{Volume taken for estimation}}$$

(μg/mg fresh wt. of larvae).

RESULTS

The mean proline content during the larval development of *S. brockii* under different salinities along with 't' values and ANOVA are presented in Fig. 1 and Table 1.

TABLE 1. *Analysis of variances for the differences of proline content of S. brockii at various salinities.*

Source	Sum of Square	D.F.	Mean Square	F
I Zoea				
Main effect	8085.471	5	1617.094	
Deviation	1665.255	24	69.386	23.3057*
II Zoea				
Main effect	29204.437	5	5840.887	
Deviation	4796.000	24	199.833	29.2287*
III Zoea				
Main effect	617.970	5	123.594	
Deviation	610.826	24	25.451	4.8561*
IV Zoea				
Main effect	548.420	5	106.684	
Deviation	540.847	24	22.535	4.8672*
Megalopa				
Main effect	540.111	5	108.022	
Deviation	432.029	24	18.001	6.00083*

* Indicate the satistically significant differences from control value ($P < 0.05$).

DISCUSSION

It is well established from the present study that the content of proline increased with increasing salinity during the larval development of the mangrove crab, *S. brockii*. Similar reports have been made by several researchers. Florkin and Schoffeniels (1960) compared the level of free amino acids (FAA) in the euryhaline marine decapod crustacea *Eriochens sincerus* cultured in a concentrated and in a diluted medium and they found that there was a remarkable accumulation of FAA in *E. sincerus* under concentrated medium. Camien *et al.* (1951) also found that the FAA level varies in marine and freshwater crustaceans with the environmental salinity. Schoffeniels and Gilles (1970) found that the FAAs regulate osmolarity of the hemolymph with changing environmental salinity. This aspect of osmoregulation was also suggested by Florkin (1956). Gerard and Gilles (1972) found that there was a decrease of the FAA pool in muscles of *C. sapidus* upon acclimatization from 100% sea water to 50% sea water, indicating the participation of amino acids in the regulation of the osmotic fluid. A similar trend was also noted in the hepatopancreas, gill and nerve tissue. The non-essential amino acids (proline) play a more important role than the essential ones in the regulation of cellular osmotic pressure (Huggins and Munday, 1968).

The possible metabolic causes for proline accumulations as reported by Kathiresan (1987) are as follows: (a) decreased proline utilization due to decreased protein synthesis and slow imcorporation of proline into protein, (b) lowered rate of proline oxidation, (c) de novo synthesis of proline from glutamate or from arginine and (d) increased proteolysis during stress. Florkin and Schoffeniels (1965) suggested that the changes in the level of amino acids cannot be attributed to a simple dilution of sea water but to the changes of cellular volume.

It was suggested by many authors that proline accumulates to counteract the saline stress-induced effects. However, a few authors hold a strong view that proline accumulation is nore a symptom of stress rather than an adaptive biochemical modification. According to Chaplin *et al.*. (1966) the amino acids serve

as a source of energy and nitrogen during starvation of *Artemia* under salinity stress conditions. Involvement of the amino acids in crustacean osmoregulation during salinity stress was suggested by Schoffeniels (1960). Florkin

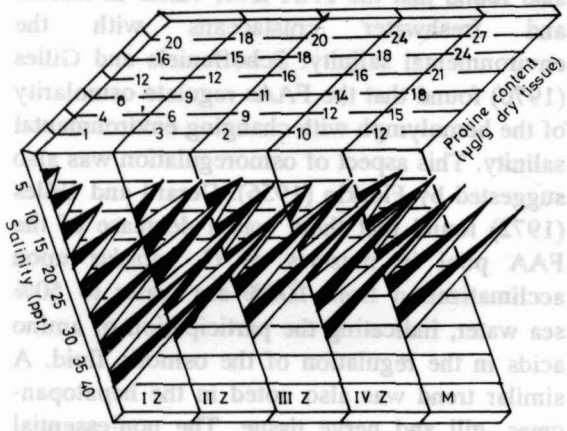

FIG. 1. *The mean proline content during the larval development of S. brochii*

and Schoffeniels (1965) and Camien *et al.* (1951) found that amino acids such as proline, glycine and arginine were highly concentrated in muscles of crustaceans, while aspartic acid was more in nerve and alanine and glutamine in the blood due to saline stress.

In the present study, the first zoea cannot survive more than an hour in 5 and 40 ppt salinities. The proline content was 0.989 µg/mg in 40 ppt in first zoea (Fig. 1). Although there was a significant difference in the content of proline between 5 and 40 ppt, there was no survival of first zoea in either of the salinity. Hence, it is inferred that the proline accumulation does not play any role in the survival of the first zoea under extreme saline stress and the proline accumulation is only an after-effect of saline stress.

Many workers recorded the accumulation of amino acids in developing organisms. The proline was most abundant in the hatched nauplius of *Artemia* (Yamaoka an Scheer, 1970). Emerson (1967) found that the amino acid content was dependent upon the embryonic development as well as on external salinity. During development, the amino acids such as alanine, glycine, threonine, histidine and lysine were increased. If *Artemia* was incubated in distilled water, the total amino acid concentration declined during development. In either 0.5 M or 1.0 M sodium chloride, total amino acid content was increased. In the *C. sapidus* and *R. harrisii*, the relative concentration of FAA was the highest in the eggs, decreased in the first zoeal stage and increased during the first crab stage of *C. sapidus*. But there was no definite pattern of FAA observed during the development of *R. harrisii* (Costlow and Sastry, 1966). In the present study the trend of proline accumulation was similar to that of *C. sapidus*.

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EFFECTS OF SOME ENVIRONMENTAL PARAMETERS ON RESPIRATORY METABOLISMS OF *DOTILLA MYCTIROIDES* (M-EDWARDS) OF MITHBAV CREEK

ABSTRACT

The study deals with the rate of oxygen consumption in *Dotilla myctiroides* of Mithbav Creek during the period 1991-92. A dense population of these crustacean has high rate of tolerance. The rate of oxygen consumption uptake was increased along with lowering the salinity. The rate of oxygen uptake did not change between 28 - 32.5‰. Below normal pH of 7.4 the rate of oxygen uptake was increased but above it decreased. The rate of oxygen consumption increased stepwise with the rise in temperature from 25 to 37°C, but decreased sharply between 38°C to 40°C.

SEVERAL environmental parameters are known to influence the respiratory metabolism of crabs. The rates of oxygen uptake under various environmental conditions are well documented by many investigators in estuarine shellfishes (Read, 1962, Helm and Trueman, 1967, Bayne, 1971, Deshmukh, 1972, Mane, 1975). Many other workers like Rao (1958) and Gopalakrishnan (1957) have shown that temperature is the major controlling environmental factor influencing the metabolic rates and rate of oxygen consumption. The present investigation was undertaken to study the influence of various environmental factors on the rate of oxygen uptake in *Dotilla myctiroides* and the variation in the respiration in relation to starvation and diurnal rhythm. The estuarine soldier crab is very much sensitive to these drastic changes especially the respiration and osmoregulation.

MATERIAL AND METHODS

The crabs *D. myctiroides* were collected from Mithbav creek (Lat. 16° 20'N; Long 73° 25'E) at Sindhudurg district, on the west coast of India. The crabs were brought to the laboratory, cleaned to remove the mud and sand particles and were kept in larger aquaria containing sea water (32.5‰). The test salinities were approximated with the collection salinities over the crab beds. The animals were kept for 2-3 days for acclimatization in laboratory.