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BIOCHEMICAL CHANGES DURING LARVAL DEVELOPMENT OF MANGROVE CRAB *METAPLAX ELEGANS* (DE MAN)

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ABSTRACT

The biochemical components such as total protein, carbohydrate and lipids were measured during larval development of the mangrove crab *Metaplax elegans* from first zoea to first crab stage. Protein content per larva increased from first zoea to fifth zoea, but decreased in megalopa, again increased in first crab stage. Carbohydrate content increased from first zoea to third zoea, decreased in fourth and fifth stage, and increased in megalopa and first crab stage. The total lipid shows upward trend from first zoea to first crab.

INTRODUCTION

THE INFORMATION on biochemical changes during larval development is available for only temperate waters (Frank *et al.*, 1975; Sulkin *et al.*, 1975; Holland, 1978; Kannupandi, 1980; Amsler *et al.*, 1984; Amsler and George, 1984). But similar studies have not been carried out in tropical waters in general and Indian waters in particular. Hence it is attempted in the present study to investigate the total protein, carbohydrate and lipid during larval development from first zoea to first crab of a Mangrove crab *Metaplax elegans* which has five zoeal and one megalopa stages before attaining first crab stage (Pasupathi and Kannupandi, 1988).

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MATERIALS AND METHODS

The ovigerous females of *M. elegans* were collected from Pitchavaram mangroves (11° 29'N; 79°49'E) and were kept in a plastic trough containing filtered seawater till larval hatching. After hatching thirty larvae per fingerbowl were transferred (Salinity 25 ± 1‰ temp. 29 ± 1° C). Larvae were fed with freshly hatched greatwall band (PR china) *Artemia* nauplii.

Total protein, carbohydrate and lipid were analysed from first zoeal stage to first crab stage. Ten mg of fresh larvae from each stage was used throughout the study for protein and carbohydrate and fifty mg larvae for the total lipid estimation, because of gravimetric method (The total number of larvae present in 10 mg and 50 mg is given in Table 1). Immediately after weighing, larvae were washed with deionised water, dried on filter paper and then analysed in the Hitachi double beam Spectrophotometer.

For the total protein, the method of Raymont *et al.* (1964) was followed. For the total carbohydrate, the procedure of Dubois *et al.* (1956) was adapted. For total lipid estimation the method of Folch *et al.* was followed. Standards were prepared for protein and carbohydrate from Bovine serum albumin and dextrose sugar respectively. Using these standard values, a value for μgm protein and μgm carbohydrate per larva was calculated for each sample.

Results

There is general upward trend in increase in fresh weight of larvae from first zoea to first crab stage. The increase in larval weight is directly proportional to that of decrease in larval number. The highest rate of weight is increased during megalopa to first crab stage.

Growth in terms of total protein, carbohydrate and lipid contents per larva was calculated and presented in Table 1.

Table 1. *Variation in biochemical composition during larval development of* Metaplax elegans

Stages	Total larvae per 10 mg	Total la.vae per 50 mg	μgm protein per larva	μgm carbohydrate per larva	μgm lipid per larva
I	390	2000	11.76	7.32	6
II	275	1475	16.19	9.30	8.80
III	230	1217	19.95	10.96	9.86
IV	183	980	27.64	9.41	11.73
V	129	630	46.74	8.79	19.84
Megalopa	76	345	43.34	49.31	39.13
First crab	36	195	98.04	70.74	97.44

The amount of protein per larva generally increased from first zoeal stage to fifth zoeal stage. Then the value was decreased in megalopa stage and once again increased in first crab stage. Overall about 8.34 times increase in protein content in first crab, when compared to first zoea stage. The highest accumulation of protein was during megalopa to first crab stage. Table 2 shows the percentage protein increased per larva for each stage. The percentage increase in protein value also dropped from fifth stage to megalopa. The greater percentage of protein usually acts as the main energy source during larval development.

Unlike protein, the carbohydrate content was always less for each stage. The carbohydrate content was steadily increased from first stage to third stage, then it steeply decreased in fourth and fifth stages, and once again increased in megalopa and first crab stage. The carbohydrate content increased 10 folds from first zoea to first crab. The indirect relation is between carbohydrate and protein content during fifth stage larva to megalopa stage *i.e.* when the carbohydrate content increased from fifth stage to megalopa at the same time protein value decreased.

The total lipid content per larva increased steadily from first stage larva to first crab stage. Compared to protein and carbohydrate, low amount of lipid was present in the early stages (first-third stage). Percentage increase in lipid value was less from second to third and third to fourth stage larva. Overall, about sixteen times of lipid content increased from first stage larva to first crab. The highest percentage of lipid increased from megalopa to first crab stage. In first crab stage, the lipid value was very similar to that of protein value.

Discussion

The present study clearly indicates that there is general increase in protein content from first zoeal stage to fifth zoeal stage (Table 1). Similar observations have been reported for protein content and protein pattern during larval development of *Rhithropanopeus harrisi* (Frank *et al.,* 1975, Kannupandi, 1980) and *Hyas araneus* (Anger *et al.,* 1983). The increased trend in protein content in the present study from first zoea to fifth zoea is attributed to increased protein synthesis during larval development. In this context, it would be relevant to recall the observations of Sulkin *et al.* (1975) who have shown that total DNA and RNA content increased from first zoea to 4th zoeal stages of *R. harrisi* reflecting maximum protein synthesis activity. Decrease in protein content from 5th zoea to megalopa can be attributed to the critical changes taking place in shape and as well as in organisation, prior to metamorphosis (Kannupandi, 1980). Further it is interesting to note that the post larvae megalopa utilizes 5.68% of stored protein from the fifth stage (Table 2). It is suggested that the megalopa requires more protein rather than lipid. This suggestion gains support from the study of the megalopa of *Callinectes sapidus* by Holland (1978), which utilizes more protein rather than lipid as an energy substrate. Again the maximum amount of protein in the first crab stage is indicative of an active protein synthesis (Kannupandi, 1980).

Unlike protein content, the carbohydrate content in early stages was less. Perhaps, the carbohydrate may be a minor contributor to embryonic metabolism and appears to be a minor energy substrate in early development (Holland, 1978). In the present study, from third zoeal stage to fourth and fourth to fifth stages, the carbohydrate utilization was higher when compared to other stages. In these stages, the additional carbohydrate is not added, but some percentages (Table 2) of stored carbohydrate in the previous stages, is utilized by the larvae as in the case of lobster *Homarus americanus* (Cappuzo and Lancaster, 1979), where the carbohydrate content decreased from third larval stage to postlarval stage. When the carbohydrate content increased from fifth stage to megalopa, the protein content decreased, suggesting that the megalopa requires more protein than carbohydrate and lipid. Perhaps the increase in carbohydrate content in first crab stage indicates its involvement in chitin synthesis, which requires the structural carbohydrate glucosamine (Amsler and George, 1984).

TABLE 2. *Percentage increase in biochemical composition during larval development of* Metaplax elegans

Stages	Protein increased per larva	Carbohydrate increased per larva	Lipid increased per larva
I-II	45.16	20.18	28.54
II-III	52.59	23.22	14.83
III-IV	72.89	-14.69	17.73
IV-V	74.55	-2.42	31.65
V-Megalopa	-5.68	67.75	32.25
Megalopa-First crab	42.46	16.63	45.26

In crustacean larvae, the lipid is particularly an important component in maintaining the structural and physiological integrity of cell membranes (Connor and Gilbert, 1968). In early larval stages, comparatively low lipid values may reflect considerable utilization of lipid reserves during embryonic development, prior to hatching. Similar observations have been reported for the total lipid content during larval development of *R. harrisi* (Frank *et. al.*, 1975; Kannupandi, 1980) and *Hyas araneus* (Anger *et al.*, 1983). A marked net accumulation of lipid during larval development noticed in the present study suggests that the larvae utilize an increasing percentage of consumed energy for tissue production, as suggested by Mootz and Epifanio (1974), during larval development of the stone crab *Menippe mercenaria*. Lipids accumulated in the later stages is utilized for metabolic energy production as well as for cellular and sub-cellular activities in preparation for metamorphosis. In addition it is interesting to note that increased lipid is generally coincided with the period of high cell multiplication (Frank *et al.,* 1975). In later stages to meet the metabolic requirements for lipids, the larvae must either synthesize or receive them via diet. It has been shown by Whitney (1969), the larvae of *R. harrisi* are not capable of synthesizing the sterols, which is important for moulting. In the present study yolky nauplius of *Artemia* used as diet, which apparently fulfils the dietary requirement for successful larval moulting and development of *M. elegans.*

Biochemical changes observed in the present study can be attributed not only to growth during larval development, but also due to complex contribution of factors which occur in a cyclic fashion, such as water uptake and which vary according to changes in cell size and number (Passano, 1960). Subsequently, the tissue production and accumulation of organic reserves are achieved only after increase in size. The changes in biochemical components, such as protein, carbohydrate and lipids, in the present study, show that these changes are relevant indicator of growth during larval development than either size increase or moult frequency.

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