

MOLT RELATED BIOCHEMICAL CHANGES IN FRESHWATER PRAWN, *MACROBRACHIUM MALCOLMSONII* (H. MILNE EDWARDS)

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Abstract: Biochemical changes which occur during the molt cycle of freshwater prawn, *Macrobrachium malcolmsonii* and the characteristic features of the five molt stages from A to E are described in this article. In abdominal muscle, the carbohydrate level decreased significantly ($P < 0.05$) from 18.38 ± 0.91 mg/g in stage A to 17.56 ± 0.53 mg/g in stage B, and then increased significantly ($P < 0.05$) to 20.68 ± 0.74 mg/g in stage D. Though, the maximum protein level was recorded in stage D (71.6 ± 1.89 mg/g) and the minimum level was noticed in stage B (56.51 ± 1.29 mg/g) ($P < 0.05$). The lipid level reflected the same trend with the maximum in stage D (75.44 ± 0.72 mg/g) and the minimum in stage B (54.88 ± 0.25 mg/g) ($P < 0.05$). The total mineral content of the muscle was at the maximum level in stage C (65%) and the minimum in stage E (23%) ($P < 0.05$). Of the three minerals, in muscle and exuvium, the calcium level (17.4%) is always higher than in the latter. Meanwhile between all the molt stages, the amount of potassium and sodium is higher in stage D. Variation in the moisture content reported in the study is due to the accumulation of organic materials. Similarly, increased ash content from stage A ($4.5 \pm 0.5\%$) to stage E ($11.8 \pm 1.2\%$) ($P < 0.05$) is also due to the outcome of mineral accumulation.

Keywords: *M. malcolmsonii*, Molting, Biochemical

INTRODUCTION

Indian river prawn *Macrobrachium malcolmsonii*, which is the second largest freshwater prawn, has received wide attention due to its culture potential matching with that of giant freshwater prawn *M. rosenbergii*. Available information on *M. malcolmsonii* essentially pertains to culture and larval rearing (Kanaujia *et al.* 1997). Studies on biochemical variations in *M. malcolmsonii* due to extrinsic and intrinsic factors are an open avenue for research. These variations are highly influenced by a number of factors like molting, reproductive stage, season, etc. Diecdysic crustaceans molt event enables animal to grow and regenerate the lost appendages.

The most prominent process dominating the life of crustaceans is molting. Metabolism, reproduction and behaviour are affected both directly and indirectly by the periodic shedding of the exoskeleton (Passano 1960). The present study aimed to emphasis on the biochemical changes during the molt cycle of freshwater prawn *M. malcolmsonii*.

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MATERIAL AND METHODS

Juveniles of *M. malcolmsonii* (1590 ± 325 mg) were collected from river Cauvery, South India and stocked in large rectangular cement aquaria ($90 \times 60 \times 60$ cm; 175 L capacity) with adequate aeration. Experimental animals were reared individually in aerated plastic aquaria (16 L capacity) under normal laboratory conditions (12L: 12D; $28 \pm 2^\circ\text{C}$). Animals were fed *ad libitum* with commercially available feed (Magnum Scampi feed; 31% crude protein, 4% crude fat, 7% crude fiber and 11% moisture) twice a day (0800 h and 1800 h) and observed regularly for molting. The five molt stages A, B, C, D and E were identified by using modified formula of Drach (1944) and Peebles (1977). *M. malcolmsonii* ($n = 20$) was carefully examined the pleopods, each under microscope.

Crude protein, moisture and ash content in each stage were quantified (AOAC 1995), as well as carbohydrate (Roe 1955) and total lipids (Folch *et al.* 1957). Total minerals (Welinder 1974), calcium, sodium and potassium were estimated following the method of AOAC (1995).

Statistical Analysis

Studied parameters like carbohydrate, protein, lipid, moisture, ash, total minerals, calcium, sodium and potassium were analyzed through one-way analysis of variance.

RESULTS

A systematic explanation of the different molt stages of *M. malcolmsonii* is given in Table 1. Stage A starts when the animal casts off the old exuvium. In this stage, the exoskeleton is soft, parchment like and uncalcified. The epidermis is transparent. Stage B, which begins with the onset of calcification is distinguished with an increase in the concentration of pigments in epidermis and formation of internal cone in each seta of uropod. Calcification process continues and internal cone formation is completed in stage C. During stage D which is the only lengthy period occupying more than 70% of the intermolt period, invagination of epidermis occurs and new setae develop. A space is formed between the old and the new cuticle. Stage E is denoted by shedding of exuvium from the animal, which lasts for a few seconds ($< 1\%$ of intermolt period).

Changes in moisture, ash and total mineral content of different molt stages of *M. malcolmsonii* are seen in Figure 1. In freshly molted prawn (stage E), the percentage of moisture was 81.83 ± 2.87 which decreased to 77.87 ± 0.51 in stage A ($P < 0.05$), then further decreased to 71.71 ± 0.10 in stage C ($P < 0.05$) and increased to 78.04 ± 1.4 in stage D ($P < 0.05$). Like moisture, the ash content also increased in values in postmolt and premolt period.

Table 1: *M. malcolmsonii*: Molt stage characterization.

Stage	Identification characters
A	Exoskeleton very soft, epidermis transparent and internal cone of uropod absent.
B	Hardened exoskeleton, epidermis granular and internal cone begin to form in uropod.
C	Hard exoskeleton. Formation of internal cone completed.
D	New cuticle appears. Space between old and new cuticle formed. New setae found in uropod.
E	Old cuticle shed within less than 2 min. Animal more sensitive to stress.

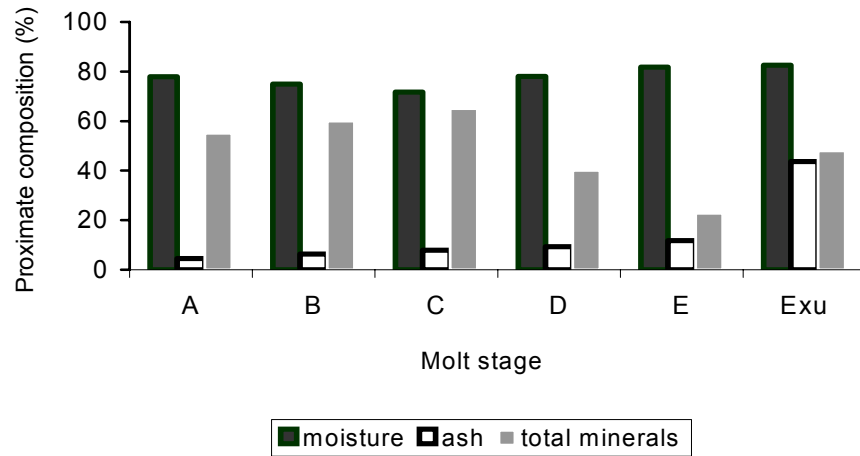


Figure 1: *M. malcolmsonii*: Changes in moisture, ash and total mineral content (wet weight in %) of muscles and exuvia (Exu).

Total mineral content, which was higher in the postmolt stage decreased during the intermolt stages D and E. Of the studied, mineral calcium shows a higher percentage in exuvia. In muscle, potassium and sodium content is higher in stage D than in exuvia (Fig. 2).

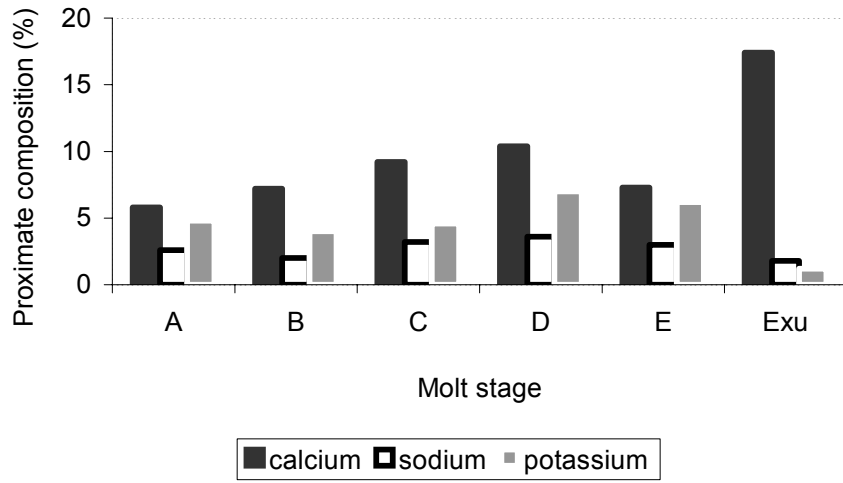


Figure 2: *M. malcolmsonii*: Changes in calcium, sodium and potassium content (%) of muscle and exuvia (Exu).

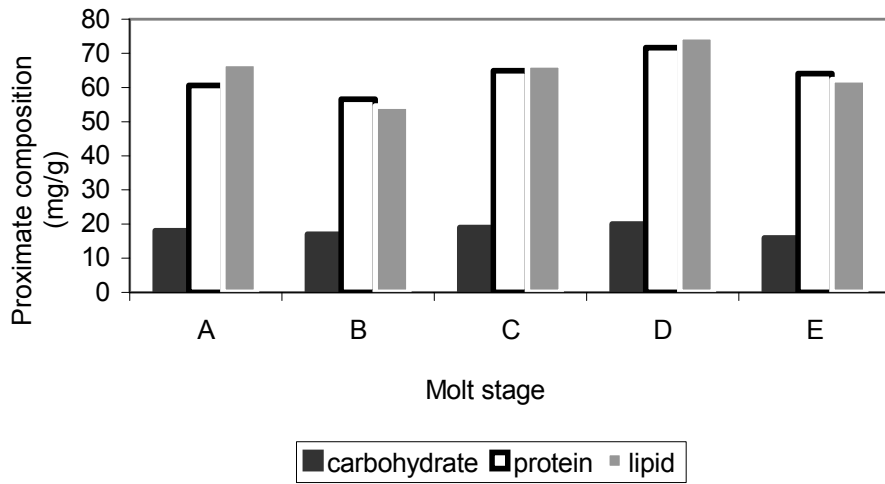


Figure 3: *M. malcolmsonii*: Changes in carbohydrate, protein and lipid content in wet weight (mg/g) of muscle.

Changes in the macro nutrients like carbohydrate, protein and lipid in abdominal muscle of *M. malcolmsonii* are seen in Figure 3. In stage A, the carbohydrate level in the abdominal muscle was 18.38 ± 0.91 mg/g, which was increased not significantly to 20.68 ± 0.74 mg/g in stage B ($P > 0.05$). The low amount of protein was recorded in stage B (56.51 ± 1.29 mg/g), and increased to

71.60 ± 1.89 mg/g in stage D ($P < 0.05$) when the molt cycle was progressed, but decreased during the next stage E ($P < 0.05$). A similar trend was also observed in lipid. The lipid level was highest in stage D (75.44 ± 0.72 mg/g). In freshly molted animal, lipid content falls down to 64.02 ± 3.25 mg/g ($P < 0.05$).

DISCUSSION

The biochemical changes of the molt cycle of freshwater prawn *M. malcolmsonii* were thoroughly studied. In decapod crustaceans, during molting, organs like muscle and hepatopancreas are the major storage sites for organic and inorganic nutrients. Various biochemical changes are associated with molting (Chang 1995). Water plays a major role during molting since the hydrostatic pressure casts off the old cuticle. Changes in the moisture content in the present study are in support of earlier findings of crayfish *Astacus astacus* (Huner *et al.* 1990). Drach (1939) reported that decapods imbibe water from the surrounding medium soon after molting. A significant reduction in moisture level is due to the accumulation of organic materials to meet the escalating energy demand and synthesis of new tissues for growth (Huner *et al.* 1990).

About ten minerals are listed as essential for aquatic animals; among these, calcium, phosphorus, potassium and magnesium are recommended for supplementation in crustaceans diets (Davis 1991). Calcium is the most studied mineral in crustacean biochemistry since it plays a major role in the hardening of exoskeleton that is shed regularly to allow the animal to grow (Greenaway 1985). Such an accumulation of calcium is also observed in the present study. The higher content of potassium and sodium in stage D of the *M. malcolmsonii* provides the specific flexibility and hardness of the exoskeleton.

Accumulation of organic materials during the intermolt and the premolt period has been reported for various species of decapod crustaceans (Chang 1995). Stewart *et al.* (1972) reported that in *Homarus americanus*, accumulation of glycogen occurs in hepatopancreas continuously during intermolt period, while McWhinnie and Scheer (1958) and McWhinnie and Saller (1960) found that there is no changes in blood sugar level in relation to molting, though accumulation of carbohydrate in hepatopancreas and muscle is general.

Changes in the level of protein during molt cycle have been studied in various decapod crustaceans (Chang 1995). Vigh and Dendinger (1982) reported that in blue crab, *Callinectes sapidus*, the level of cuticle protein increased to 5.7 ± 0.15 mg/cm² from 2.2 ± 0.16 mg/cm² after 10 days of postmolt. This is due to the synthesis of protein and its incorporation in cuticle. Durliat and Vranck (1982) observed an increase in protein level in stage C4 and an decreased during early stage D in crayfish, *Astacus leptodactylus*. Various types of proteins with electrophorograms indicate that in *A. leptodactylus*, the protein level attains a peak in stage C and D (Durliat & Vranck 1982). In *M. malcolmsonii*, a high level of protein accumulation is also reported in stage C. Increased protein level in premolt stages C and D is due to the accumulation of reserves between successive ecdysis, which is referred to as "storage for molting" for its subsequent use as a source of energy for ecdysis (Durliat & Vranck 1982).

Changes in the pattern of lipid accumulation in premolt prawn were reported by Ando *et al.* (1977). Travis (1955) reported that in *Panulirus argus*, the lipid content of the hepatopancreas was highest during early postmolt. Earlier in 1949, Renaud demonstrated an increase in the hepatopancreatic fatty acids, phospholipids and cholesterol in *Cancer pagurus* during early postmolt with a decrease in lipid fractions in ecdysis. A contrast report was recorded in prawn, by incorporation of ¹⁴C acetate into the whole animal (Patrois *et al.* 1978). The reason for such a divergent result is yet to be disclosed (Chang 1995). In *M. malcolmsonii*, the lipid content increased in the premolt stage D. This may be due to the active feeding of the animal (Vonk 1960), which increased rate of lipid synthesis and absorption of dietary lipid (O' Connor & Gilbert 1969).

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