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ABSTRACT

Objective: The objective of the present study was to evaluate the seasonal changes in the biochemical components viz., Proteins, lipids and carbohydrates present in the tissues of fresh water mussel *Parreysia corrugata* of Lower anicut reservoir, Tamil Nadu, India.

Materials and Methods: The live *P.corrugata* with the average body weight (ABW) of 20-25 gm each of from the lower Anaicut reservoir of was collected for a period of 24 months from April 2012 to March 2014. Protein was estimated following the method of Lowry *et al.*(1951). Lipid was estimated by the method of Bligh and Dyer (1959) and the carbohydrate was estimated of Dubois et al., (1956).

Results: Protein was the most dominant biochemical constituent in the present study. Significantly a higher level of protein was noticed during pre and post monsoon season and which was ranged between 34.6% to 61.0% respectively in the tissue of *P.corrugata*. In the present study lipids were found to be 4.30% to 8.80% in *P.corrugata*. Carbohydrates are second major biochemical content in freshwater mussels. Here the carbohydrates were ranging between 12.50%-43.99% in *P.corrugata*. Carbohydrates were found to be increased during monsoon and winter season.

Conclusions: In the present study, fluctuations in the levels of protein, carbohydrate and lipids content in all the seasons due to storage and utilization of the few organic constituents have been closely linked to complex interaction between food supply and temperature and between growth and reproductive cycle.



Keywords: Macronutrients, Freshwater mussel, Parreysia corrugata, Lower Anaicut Reservoir.

INTRODUCTION

Freshwater mussels are ecologically important group of burrowing organisms in aquatic ecosystems. The mussels are ecologically important because of their widespread distribution and biological filtration activity and also economically used as food and in the production of freshwater pearls. The knowledge of biochemical composition of any edible organism is extremely important since the nutritive value is reflected in its biochemical contents. The biochemical composition in the molluscs has been mainly studied to assess the nutritive status and also to supplement information on reproductive biology. The mussels provide high quality protein with all the dietary amino acids for maintenance and growth of human body¹. Freshwater mussels contain significant amount of omega 3 fatty acids in particular Docosahexaenoic acid (DHA). The fat of mussels contains approximately 20-28% of calories of energy. The bivalves have been subject of intense of studies despite the presence of rich diversity of edible and commercial species of India.

The overall change in the biochemical composition during an annual cycle has been correlated to the events of the gonadal cycles of organisms. A good

amount of information is available on biochemical composition of bivalve molluscs from various parts of the world^{2,3,4&5}. Glycogen is the primary energy store in bivalves ⁶ and the relative amount of glycogen stored in bivalve tissue is considered as a good indicator of body condition ^{6&7}.

Marine bivalves indicated that seasonal cycle of energy storage and biochemical cycles are closely related to reproductive activity ⁸. According to Gabbott , seasonal metabolic activities in molluscs result from complex interactions among food availability, environmental conditions, growth and gametogenic cycle ⁹.

Of all the components, changes in carbohydrates play an important role in the seasonal variation of the chemical composition. In general, the water content of the tissue of bivalves usually gives an indication of the time of spawning. Variation in dry tissue weight of molluscs is always associated with biochemical components. Seasonal changes in the biochemical constituent are the characteristics of the seasonal activities of bivalves.

In general, energy is stored prior to gametogenesis, when food is abundant, in the form of carbohydrate, lipid and protein. The particular importance of these substrates, where they are stored and the timing of their use varies among species, as well as among populations of the same species ¹⁰. Bivalves generally store

carbohydrates in large amounts during their growing season and use them over the rest of the year ¹¹; although proteins may be an energy reserve in some bivalve species ^{12 &13}.Lipids have been reported to function most importantly as energy storage substances and physical properties of biological membranes ¹⁴. Lipid accumulates in the developing gonads and depletes during spawning.

Numerous studies are present on marine bivalves at large, only a few studies have been done on freshwater forms. Lipid composition and storage strategy in molluscs, particularly of bivalves and gastropods, have been studied since lipids constitute a major fraction of molluscan tissues ¹⁵. Lipid composition and metabolism have been extensively studied in marine bivalves; a few investigations have been done on freshwater forms ^{16&17}.

The biochemical composition in the molluscs has been mainly studied to assess the nutritive status and also to supplement information on reproductive biology. The overall change in the biochemical composition during an annual cycle has been correlated to the events of the gonadal cycles of organisms.

Significantly, proteins, lipids and carbohydrates variations are related to reproductive cycle of bivalves. This shows the nutrient is one of the most important energy sources of mussels ¹⁸. Accumulation of carbohydrates generally takes place in large amounts during their growing season and uses them during



their rest of the life; proteins may also be an energy store in some bivalve species ^{19&20}.

The objective of the present study was to evaluate the seasonal changes in the biochemical components viz., proteins, lipids and carbohydrates present in the tissues of fresh water mussel *Parreysia corrugata* of Lower anicut reservoir, Tamil Nadu, India.

MATERIALS AND METHODS

The live *P.corrugata* with the average body weight (ABW) of 20-25 gm each of from the lower anaicut reservoir of was collected for a period of 24 months from April 2012 to March 2014. They were hand-picked and brought to the laboratory alive. They were identified based on the literature available. The whole body meat was isolated, blotted to remove the excess water and dried in the hot air oven at 600C till constant weight was obtained. All tissues were ground into fine powder using mixer grinder and were used for the further biochemical evaluation such as proteins, lipids, carbohydrates and ash contents.

Protein

Protein was estimated following the method of ²¹. To a 10mg of sample 1 ml of 1N NaOH was added and kept in boiling water bath for 30 minutes for protein

extraction. Thereafter, it was cooled to room temperature and neutralized with 1 ml of 1N HCL. The extracted sample was centrifuged at 2000 rpm for 10 minutes, and an aliquot of the sample (1 ml) was further diluted with

Distilled water (1/9 v/v). From the diluted sample, 0.5 ml was taken and made up to 1 ml with 0.1N NaOH. To this, 5 ml of mixed reagent (alkaline copper reagent) and 0.5 ml of FC reagent was added. After 30 minutes, O.D. was read at 660 nm using spectrophotometer.

Lipid

LISEAS

Lipid was estimated by the method of Bligh and Dyer $(1959)^{22}$. 50 mg of dried tissue sample was mixed well with 15 ml of chloroform-methanol mixture (1:2 v/v) and 4ml of distilled water. The homogenate was centrifuged at 2000 rpm for 10minutes. The supernatant was taken in separating funnel and 5ml each of distilled water and chloroform was added and mixed well and left for overnight separation. The lower layer was collected in pre weighed ceramic bowl, dried in nitrogen stream and weighed.

Carbohydrates

The carbohydrate was estimated of Dubois et al., (1956). 20 mg of dried tissue was taken and to this, 1ml of glass distilled water was added followed by 1



ml of 5% phenol solution and 5 ml of concentrated sulphuric acid one after another. After 30 minutes, Calorimetric reading was taken in Hitachi double beam spectrometer at a were length of 490 nm against blank reading.

Results

Protein

Protein was the most dominant biochemical constituent in the present study. Significantly a higher level of protein was noticed during pre and post monsoon season and which was ranged between 34.6% to 61.0% respectively in the tissue of *P.corrugata*. The highest values of protein content in *P. corrugata* was recorded during October (61.00% & 66.00%) and the lowest during February (34.60% & 38.00%) in the study period.

Lipid

Lipid is an important dietary constituent serve as energy when food supply is scantly. In the present study lipids were found to be 4.30% to 8.80% in *P.corrugata* and the highest value was recorded in lipid content decline in winter indicate that at the time of fully maturity of gonads the other biochemical content increased and lipid content lower and increased in post monsoon due to ripening and matured released of gametes. On the other hand specific differences in lipid



composition in mussels are also affected by its diet, vegetation, environmental temperature, salinity and dirtiness of water. Most importantly diet plays a major role in it.

Carbohydrates

Carbohydrates are second major biochemical content in freshwater mussels. Here the carbohydrates were ranging between 12.50%- 43.99% in *P.corrugata*. Carbohydrates were found to be increased during monsoon and winter season. The glycogen content in the gonads, decline in monsoon season in mussel. Might may be due to starvation, reproductive stages and drasic environmental conditions and low metabolic rate. In the winter the glycogen content increases in the gonads. Discussion

The study evaluate the seasonal variation of stored biochemical constituents such as protein, lipid and carbohydrates in freshwater mussel *P.corrugata* revealed an increasing trend in the biochemical constituents from premoon soon period. This information is basic for understanding the seasonal variation of biochemical constituents of this species.

Although glycogen is regarded as major for of energy reserve in bivalves ²³, protein reserve may be used simultaneously with carbohydrates, or even as the primary energy source^{24&25}.

The protein maxima and minima correspond to the development / spawning/and regression/ resting phases, respectively ²⁶. This could be mainly because of increase food availability and secondly it happened to be just prior to the spawning period. This increased protein content may be a mechanism of maturation of gonad and storage of reserves to meet spawning requirements ^{27&28}. Low protein values recorded in monsoon were could be mainly due to increased rate of ammonia excretion and also spawning season which are in conformity with earlier reports ²⁹.

In stress environmental conditions, after glycogen lipid is used as energy source ^{30&31}. Lipid variation has been related to gamete development with the highest levels of lipids accumulation during the gonadal ripening. Lipids also exhibited large fluctuations in all months of the year.

But in monsoon months as temperature and salinity decreases sharply lipids also decreases sharply. The increase in lipid content may be due to the lipogenesis occurring in the ovary for production of gametes. In case of mussels lipids shows inverse relation with carbohydrates. As, lipid increases carbohydrates decreases.

Measuring of macromolecules (carbohydrate, protein, and lipid) revealed the biochemical function and energy storage in freshwater Unionids ³². Glycogen, the primary energy reserve in bivalves, drives many important physiological processes



and could be used to ensure short-term exposure to anoxia, emersion and reduced food supplies. The highest glycogen content in P. corrugata was 16.3 mg g-1 (April) and the lowest was 1.81 mg g-1 (January) and the values of glycogen increased slowly from March to May due to buildup of gonads. The fall in glycogen content from October onwards could be due to spawning activity. The gradual increased content of glycogen from March onwards could be due to the development of the gonads ³³. An intimate association of glycogen with the period of sexual activity was also observed in Corbicula sp. 32&34 observed that the glycogen content in Lamellidens margianalis was 4.94%.² in P. corrugata observed that the glycogen content varied from 4.57 to 5.73% of dry weight in various size groups. They further observed that the variation in the glycogen content was not size dependent. Histochemical preparation showed that maximum glycogen concentration was noticed in the mantle, muscles, gills, gonads and digestive diverticula of mussels². Significant reductions in Unioid glycogen greatly reduce their ability to cope with natural stressors present in the new environment. Glycogen storage fluctuates seasonally during ebb periods of gametogenisis and decrease rapidly in response to reduced food availability and environmental stress ^{35&36}.

The nutrients protein, glycogen and lipids vary seasonally. These changes are generally related to the reproductive cycle. Similar characteristics have been observed bivalves such as *Anomalocardia squamosal*, ³⁷Donax trunculus ³⁸,Lyrepecten (Nodipecten nodosus ³⁹,Macoma balthica ⁴⁰, Mercenaria mercenaria ⁴¹,Placopecton magellancius ⁴², Venus verrucosa ⁴³.







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