

BIOCHEMICAL VARIATION IN FRESH WATER BIVALVE *LAMELLIDENS MARGINALIS*

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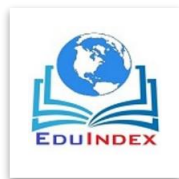
ABSTRACT

The seasonal patterns of biochemical composition in bivalve, *Lamellidens marginalis* from different habitats from the Marathwada region were observed in different seasons. As environmental factors changes it shows an effect on biochemical composition in the tissues. The bivalve mollusc shows maximum variation of biochemical composition as it undergoes different stages like development, maturation and spawning during different seasons and can be affected by environmental factors, such as fluctuations in the environmental conditions, or by internal factors, such as metabolic and physiological activities. It might be the spawning cycle and food supply are the main factors responsible for this variation. In Jayakwadi and Bindusara dam the protein level was found maximum during winter (53.50) and (48.70) mg/100gm of dry tissue weight. In Manjara and Yeldari dam it was more (47.50) and (47.10) in monsoon. There is great fluctuation in the values of glycogen were observed during different seasons. The glycogen level was found maximum during winter in Bindusara (10.39) followed by Jayakwadi (11.09), Manjara (10.34) and Yeldari (9.69). The lipid was more in Jayakwadi (6.48) followed by Manjara (6.29), Yeldari (6.29) and n Bindusara dam (6.05) in winter. The ascorbic acid was more in Jayakwadi (3.07) followed by Manjara (3.03), Bindusara (3.02) and Yeldari dam (2.94) in winter.

Keywords: Freshwater bivalves, *L. marginalis*, Protein, Glycogen, Lipid, Ascorbic acid

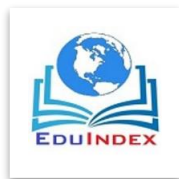
INTRODUCTION

In invertebrates changes in the biochemical constituents are pronounced which are cyclic in



reproduction, since a great amount of energy must be channelized to the gonad during reproduction. Bivalve molluscs are potential sources of valuable biochemical and are abundantly available in India. However, their abundance and diversity have declined in the last 30 years and they are now among the most imperiled groups of animals in the world. The biochemical composition of mollusc is influenced by its size, growth and reproductive status. Bivalves play an important role in the ecosystem equilibrium and constitute an important economic end point. Habitat destruction, including increased siltation, pollution, and river modification, loss of fish hosts, commercial exploitation, and introduced species, are among the causes of their decline (Bogan, 1993). The bivalves have not been the subject of intense studies despite the presence of rich diversity of edible and commercial species in India Shetty *et al.* (2013). 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 Martinez(1991). 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 Galap *et al.*, (1997) and Brockington (2001).

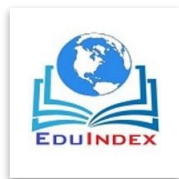
In addition, biochemical assay provide both qualitative and quantitative changes of tissue level in the bivalve. Sometime specific responses shown by, for example, fishes to certain kind of toxicants such as heavy metals pesticides are particularly useful in fishery management and resources protection (Petering and Fowler, 1986, and Suryawanshi *et al*, 2015) Kulkarni (1993) and Patil (1993) studied cadmium chloride and summer induced changes in the biochemical composition of the freshwater bivalve *L. marginalis* respectively. Devi (1996) studied bioaccumulation and metabolic effects of zinc and mercury on marine dreissinid bivalve, *M. sallei*. Munsiet *al.*(1997) studied the mixture of heavy metals on the biochemical composition of two penaeid shrimp post larvae. Reddy *et al.* (1986) studied the effect of summer on carbohydrate metabolism of the soft body parts of the freshwater bivalve *Parreysia rugosa*. Mane and Gokhale (1990) observed biochemical changes due to acute toxicity of fluoride to *L. marginalis* and found



significant changes in protein content from mantle, gill, hepatopancreas, gonad and foot. Napolitund *et al.* (1992) studied the lipid composition of egg and adductor muscle in giant scallops *P. magellanicus*. Suryawanshi *et al.* (2017) studied base levels of heavy metals in different body parts and tissue specificity of *L. marginalis*. Many researchers from Maharashtra devoted to study on the effect of various pollutants on freshwater bivalves in relation to biochemical aspects of body physiology (Andhale, 2011, Waghmare *et al.*, 2012, Waykar and Shinde, 2013, Mundhe and Pandit 2014). But, very little information is available on the base levels of biochemical in the body from Marathwada region in Maharashtra. Since limited amount of information is available on the freshwater bivalves, the present investigation has been undertaken to study seasonal variations in the biochemical composition like protein, lipid, glycogen and ascorbic acid values of bivalves *L. marginalis* from different habitats of Marathwada region during different seasons and to gather information on the uses of these bivalves as food or medicines for the local people.

MATERIALS AND METHODS

The animal habitats are rich in flora and fauna around and in as there is no any industry on both sides as well as in catchment area. The availability of bivalves for present study depend upon the topography of the dam weather condition and human activities like pollution, heavy water force, interfering the cattle, washing the cloths along the dam etc. The localities in dams are selected as per the species abundance and water qualities of dam in different geographic area. The bivalves *Lamellidens marginalis* were collected for laboratory experiments from different places of Marathwada region during different seasons. They were brought to the laboratory and kept in plastic troughs containing five liters of dechlorinated tap water for three days to acclimatize to laboratory conditions. Water from the plastic trough was changed after every 12 hours. The healthy bivalves of approximately same size and weight were selected for the experiments. Since the animals are micro feeders no special food was supplied during the experiment. The acclimatized bivalve *L. marginalis* were sacrificed to analyze the biochemical composition. The bivalves were dissected and their mantle, foot, gill and digestive gland were separated and whole body mass of remaining bivalves was taken. All tissues were dried powders of different tissues were used for

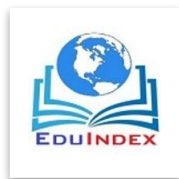


estimation of their protein, glycogen and lipid content. The amount of total protein in tissue was estimated by using Lowry's method (Lowry *et al.* 1951). Glycogen was estimated by Anthrone reagent method (Dezwaan and Zandee, 1972). Vanillin reagent method of Barnes and Blackstock (1973) was used to estimate the amount of total lipids in the tissue. The cholesterol was used as a standard for lipid estimation. Ascorbic acid from bivalves was estimated using the method Roe (1958). The amount of biochemical was obtained were statistically analyzed for confirmation of the results and expressed in $\mu\text{g/g}$ dry tissue.

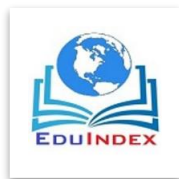
RESULTS

During present study the samples were collected during Monsoon (August), Winter (December) and Summer (April) and results (**Table-1**) showed that the protein level was more in winter i.e. (54.25),(48.70),(47.13),(45.25) in Jayakwadi, Bindura, Manjara, Yeldari respectively. Further in monsoon it was more in Jayakwadi dam(53.0), followed by manjra(47.50), followed by Yeldari(47.10) and (45.99) Bindusara. When the protein from winter and summer was compared with monsoon from the same dam, in Jayakwadi during winter it was more (1.43%) and low in summer(19.05%, $p<0.001$). Further the content was low at (20.19%, $p<0.001$) in summer when it was compared to winter. For Bindusara dam in winter it was more (6.03 %) and low in summer (11.12 %, $p<0.001$). Further in summer the content was low (16.17 %, $p<0.001$) when it was compared with winter. In Manjara dam the protein was low at (0.80 %), (17.08 %, $p<0.001$) in winter and summer respectively when it was compared to monsoon. In summer it was low (16.42 %, $p<0.001$) when compared with winter. In Yeldari dam the content was low at (4.02 %, $p<0.01$), (20.72 %, $p<0.001$) in winter and summer respectively when it was compared to monsoon. Further in summer it was low at (17.41 %, $p<0.001$) when it was compared to winter.

The glycogen level was more in winter i. e. (12.01), (11.39), (10.34) and (9.69) in Jayakwadi, Bindusara, Manjara and Yeldari dam respectively. Further in monsoon the content was more (11.0s9), (10.94) (9.07) and (8.96) in Jayakwadi, Bindusara, Yeldari and Manjara dam respectively. Whereas the content was more in summer was from Manjara (8.20) followed by Yeldari (8.0), in Jayakwadi (6.68) in and Bindusara dam (5.33). When the glycogen from winter



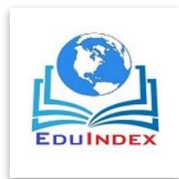
and summer was compared with monsoon from same dam, in Jayakwadi during winter it was more (9.12%, $P < 0.01$) and low in summer (43.71, $p < 0.001$). Further in summer the content was low at (48.42%, $p < 0.001$) when it was compared to winter. In Bindusara dam in winter it was more (4.53 %) and low in summer (56.44%, $p < 0.001$). Further in summer the content was low (58.33%, $p < 0.001$) when it was compared with winter. In Manjara dam the content was more (17.34 %) in winter and low (9.55 %, $p < 0.01$) in summer when it was compared to monsoon. In summer it was low (22.92%, $p < 0.001$) when compared with winter. In Yeldari dam the content was more (7.69%) in winter and low (13.26%, $p < 0.01$) in summer when it was compared to monsoon. In summer it was low (19.45%, $p < 0.001$) when compared with winter. The lipid level was more in winter i. e. (5.48), (5.29), (5.29) and (5.05) in Jayakwadi, Manjara, Yeldari and Bindusara dam respectively. Further in monsoon the content was more (5.72), (5.57) (5.49) and (5.09) in Yeldari, Bindusara, Jayakwadi, and Manjara dam respectively. Whereas the content was more in summer was from Bindusara dam (3.78), Manjara (4.66), Yeldari (4.54), in Jayakwadi (2.79). When the lipid from winter and summer was compared with monsoon from same dam, in Jayakwadi during winter it was more (22.05%, $P < 0.01$) and low in summer (37.87, $p < 0.05$). Further in summer the content was low at (49.09%, $p < 0.01$) when it was compared to winter. In Bindusara dam in winter it was more (10.51%) and low in summer (13.29%, $p < 0.05$). Further in summer the content was low (25.15%, $p < 0.05$) when it was compared with winter. In Manjara dam the content was more (29.34%) in winter and low (10.52%, $p < 0.05$) in summer when it was compared to monsoon. In summer it was low (30.82%, $p < 0.001$) when compared with winter. In Yeldari dam the content was more (12.08%) in winter and low (25.0%, $p < 0.001$) in summer when it was compared to monsoon. In summer it was low (33.09%, $p < 0.001$) when compared with winter. The ascorbic acid level was more in winter i. e. (3.07), (3.03), (3.02) and (2.94) in Jayakwadi, Manjara, Bindusara and Yeldari dam respectively. Further in monsoon the content was more (2.91), (2.90) (2.85) and (2.77) in Jayakwadi, Bindusara, Manjara and Yeldari dam respectively. Whereas the content was more in summer was from Jayakwadi (2.32), Bindusara (2.28), Yeldari (2.25) and in Manjara (2.06). When the lipid from winter and summer was compared with monsoon from same dam, in Jayakwadi



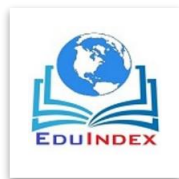
during winter it was more (8.38%) and low in summer (30.90%). Further in summer the content was low at (36.24%, $p < 0.05$) when it was compared to winter. In Bindusara dam in winter it was more (4.67%) and low in summer (33.68%, $p < 0.01$). Further in summer the content was low (36.64%, $p < 0.05$) when it was compared with winter. In Manjara dam the content was more (9.73%) in winter and low (42.71%, $p < 0.01$) in summer when it was compared to monsoon. In summer it was low (47.79%, $p < 0.01$) when compared with winter. In Yeldari dam the content was more (9.61%, $p < 0.05$) in winter and low (29.38%, $p < 0.05$) in summer when it was compared to monsoon. In summer it was low (35.57%, $p < 0.01$) when compared with winter.

DISCUSSION

In the present study evaluated the seasonal variation of stored biochemical compositions such as protein, lipid, glycogen and ascorbic acid in freshwater mussel. Generally, high and low amount of content of biochemical were found in mussels collected during winter and summer respectively, which coincide with the peak of winter and summer in this region. In the present study, fluctuations in biochemical content in all the seasons in different places 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. Gabbot and Bayne (1973) were observed 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. The results obtained in the present study are supported by several investigators who reported a decline in protein in various organisms under influence of different stressors. Torreblacaet *al.* (1991) observed that in freshwater cryfish *Procambarus clarkii* there was considerable decrease in various t issues after exposure to cadmium. Kulkarni (1993) observed increased content in the different body parts of freshwater bivalve molluscs *L. marginalis* after exposure of sublet hal concentration of cadmium. Patil (1993) also observed the protein increased from different body parts of freshwater mussel *L. marginalis* after exposure to mercury. Torreblanca *et al.* (1992) studied the changes in the biochemical composition of gills, hepatopancreas and muscle after exposure to 0.25 mg/hg/l in *P. clarkii*. They observed significant



decreases in protein concentration and caloric concentration in gills over the 96h period. Glycogen/lipid and glycogen/protein ratios increased after 48 h and 96h of mercury exposure, lipid and caloric concentration in the hepatopancreas were significantly lower and glycogen concentration in muscle was depleted as consequence of 96h mercury exposure. The results obtained in present study indicate severe disturbance in the protein metabolism of the fresh water bivalve *L. marginalis*. The results obtained in the present study are supported by several investigators who reported decrease in protein of various organisms under influence of stresses. It is in the level of tissue protein may also be due to excessive proteolysis to overcome the metabolic stress, as deposited protein in the cytoplasm can easily be used to replace the loss of proteins that occur during physiological stress. When mussels at all time period might be due to temperature variation during different seasons and hence protein was more depleted from these body when it was compared with monsoon of bivalves. It is evident that decrease in the protein in the mussels in all probably caused metabolism restricted to lipogenesis and maintenance by utilizing protein substrate. It suggests that the low level of protein level might be due to increased proteolysis activity or might be due to changes in the metabolic substrate during anaerobic condition produced in the bivalves by environmental stress. The protein levels in the body decreased continuously when increases the water temperature. The decrease of protein content, suggests possible utilization for metabolic purposes enhancement of proteolysis to meet the high-energy demand under metal pollution stress condition. The fall in the protein content during different seasons may be due to increase protein catabolism and decrease anabolism of protein. 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 gradual increased content of glycogen from summer onwards could be due to the development of the gonads Shettigar and Seetharamaiah, (2013). In present study the results showed during summer season caused some how different trend was observed, revealing different type of substrate utilization to meet the energy demand. Depletion of glycogen level might be due to the anoxia and hypoxia caused due to stress condition which is known to increased carbohydrate consumption (DeZawan and Zandee,

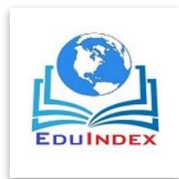


1972). Kabeer (1979) stated that decreases in glycogen content in metal exposed tissue could also be due to decreases glycogen synthesis. Decrease in glycogen content indicates disrupted carbohydrate metabolism. In bivalve molluscs the conversion of glycogen into fatty via trios – phosphate entry in glycogen sequence and to the production of pentose sugar for nucleic acid synthesis is well documented by Gabbott (1976). In the present study total lipid content of various tissues of bivalve was found to be increased during summer might be due to high water temperature. Lipids play a nutritionally and physiologically important role in bivalves by providing an efficient source of high energy content and essential fatty acids (Waldock and Holland, 1984). The nutritional composition of the bivalves can be affected by environmental factors, such as fluctuations in the environmental conditions, or by internal factors, such as metabolic and physiological activities. It might be the spawning cycle and food supply are the main factors responsible for this variation. It is well known that seasonal variations in nutritional contents of bivalves are closely linked to the reproductive cycle and climate changes and are affected by the availability and composition of the natural diet. On the basis of these results, the freshwater mussels are good source for some important nutrients such as proteins, lipids, glycogen and vitamins. Voogt (1983) stated that lipids in bivalves are multifunctional and in different species one or same of the functions during the maturation of gametes, drastic environmental conditions, starvation, population stress etc. can be more noticeable. This suggests utilization of protein and synthesis of lipid from all the body parts upon high temperature in outside medium. The study can conclude there is significant variation in the biochemical levels in the bivalves according to seasonal changes. They have got important roles in food chain since they are consumed by fish, water birds, mammals and reptiles in the river.

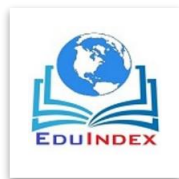
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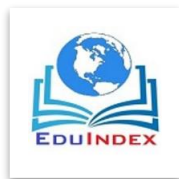
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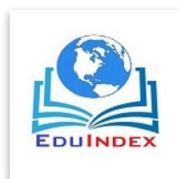
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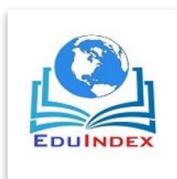


Table 1:- Biochemical changes in *Lamellidens marginalis* (wild population) from different seasons in different dams of Marathwada region.

Biochemical Constitution	Jayakwadi			Bindusara			Manjra		
	Monsoon	Winter	Summer	Monsoon	Winter	Summer	Monsoon	Winter	Summer
Protein	53.0 ±2.35	54.25 ±1.72 (1.43%)	43.50 1.65 (19.05%)□□□ (20.19%)ΔΔΔ	45.99 ±1.95	48.70 ±1.72 (6.03%)	40.99 ±0.65 (11.12%)□□□ (16.17%)ΔΔΔ	47.50 ±1.72	47.13 ±1.13 (0.80%)	39.56 ±0.22 (17.0%) (16.4%)
Glycogen	11.09 ±0.18	12.01 ±0.18 (9.12%)□□	6.68 ±0.14 (43.71%)□□□ (48.42%)ΔΔΔ	10.94 ±0.14	11.39 ±0.18 (4.53%)	5.33 ±0.12 (56.44%)□□□ (58.33%)ΔΔΔ	8.96 ±0.18	10.34 ±0.18 (17.34%)	8.20 ±0.07 (9.55%) (22.9%)
Lipid	5.49 ±0.07	6.48 ±0.25 (22.05%)□□	3.79 ±0.66 (37.87%)□ (49.09%)ΔΔ	5.57 ±0.10	6.05 ±0.04 (10.51%)	4.78 ±0.44 (17.29%)□ (25.15%)Δ	5.09 ±0.13	6.29 ±0.13 (29.34%)	4.66 ±0.07 (10.5%) (30.8%)
Ascorbic Acid	2.91 ±0.12	3.07 ±0.13 (8.38%)	2.32 ±0.35 (30.90%) (36.24%)Δ	2.90 ±0.16	3.02 ±0.10 (4.67%)	2.28 ±0.09 (33.68%)□□ (36.64%)ΔΔ	2.85 ±0.35	3.03 ±0.4 (9.73%)	2.06 ±0.17 (42.7%) (47.7%)

(Bracket values represent percentage differences) (□, Δ- P < 0. 05, □□, ΔΔ-P < 0.01, □□□, ΔΔΔ- P < 0.001, □- compared to monsoon, Δ- compared to winter of respective dams)