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SEASONAL VARIATIONS IN THE METABOLISM OF MUD CRAB *SCYLLA SERRATA* DURING NEW MOON AND FULL MOON PHASES OF LUNAR CYCLE

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ABSTRACT

Lunar cycles affect the behaviour and moulting of crustacea. Correlation between lunar cycles and physiological activities is quite well known for teleost. However, in crustacea the metabolic changes that occurred during lunar cycles are not well studied. Hence, we undertook to study the effect of lunar cycles on the body metabolism of mud crab, *Scylla serrata* throughout the year covering all the seasons namely summer, pre monsoon, monsoon, post monsoon and winter. Increased rate of excretion in the form of ammonia, urea, free amino acids and trimethyl amino oxide and the increased rate of the aquatic respiration were recorded during full moon phase over the new moon phase of the lunar cycle throughout the year. The levels of tissue biochemical compositions like total protein, total carbohydrates, total fat, free amino acid, free sugars and free fatty acid remained significantly higher in full moon phase of the lunar cycle. Our results also show a significant seasonal variation in the body metabolism which is again focused on the breeding activity of the crab. The differences in physiological and biochemical parameters during new moon and full moon phases of the lunar cycle may be associated with the moulting behaviour or the feeding activity of the mud crab, *Scylla serrata*.

Key words: Lunar cycle, Seasonal variation, *Scylla serrata* & Metabolism.

INTRODUCTION

Marine crustaceans interact with several physical factors like temperature, salinity, tidal cycles, lunar cycles etc. and these factors influence the behaviour, feeding and activity of the animals. The variation in the intermediate metabolisms in crustaceans depends upon habitat, stage in the moult cycle, reproductive state of the crabs, feeding state, food and seasonality. Akbar *et al.*[1] reported seasonal changes in biochemical composition such as water, total nitrogen, non protein nitrogen, protein, glycogen, lipid and ash content in the flesh of *Portunus pelagicus*. Oliveria *et al.*[2] reported a distinct seasonal variation in the glucose as well as glycogen concentration in heamolymph, hepatopancreas, gills and muscles of *Aegla ligulata*. Significant seasonal variation in the biochemical composition like glucose, protein, lipid and triglyceride in cray fish, *Parasaticus basilensis* was recorded by[3]. These variations were co-related with the reproductive behaviour of the crustaceans. Depletion of biochemical reserves to meet the demand for extra energy was observed during the spawning period of the crustacean. Environmental conditions, like food availability, water temperature and reproductive period appeared to be the main factors influencing the seasonal patterns of variation in the energy metabolism in crustaceans[4].

The synodic cycle of the moon i.e. lunar periodicity plays important role in the abundance of the organism which directly affects the fluctuation of the catches of commercially important marine crustacean species[5]. The studies related to lunar periodicity mainly concentrate on the pattern of locomotor, reproductive and moulting behaviour of the organism[6]. The occurrence of the endogenous rhythm implies on the

physiological mechanisms during tidal periodicity that evolved through natural selection favouring locomotion at particular states of the tide and hence lunar day[7]. Besides, it is also necessary to consider whether they are exogenous or endogenous, which environmental variable (temperature, salinity, and light period) are involved in phase setting and, more speculatively the adaptive advantage of such rhythms.

The crab fishery in India is yet to be recognized as a major fishery despite the abundant occurrence of edible crab all along the Indian coast. There are about 600 crab species occurring in Indian waters. Mud crabs, *Scylla serrata* are known for their delicious meat, nutritional and medicinal values and for export trade. Mud crabs represent the valuable component of small scale coastal fisheries in many countries of tropical and subtropical Asia, for which there has been a general trend of increased culture practice in recent years[8]. Although correlation between lunar cycles in association with annual rhythms and timing and synchronization of physiological and metabolic events is known for teleosts[9], similar information with regards to crustaceans is very rare. Hence, we aimed to study whether lunar cycle coincides with the circa-annual rhythms in terms of biochemical composition and physiological processes in mud crab *Scylla serrata.*

METERIALS AND METHODS

The mud crabs *Scylla serrata* with approximate carapace width of 5-7 cm were collected on New moon and Full moon day throughout the year from the Mondovi estuary, Panjim, Goa where salinity of the water remains 12 $\pm$ 2 PSU. Being a coastal state, Goa doesn't experience with wide fluctuation of temperature but experiences a prolonged period of rainfalls. The crabs were collected in six season namely, summer (March and April), pre monsoons (May and June), monsoons (July and August), post monsoons (September and October), pre winter (November and December) and winter (January and February). The six seasons were differentiated by taking in consideration the average rainfall, humidity and average maximum and minimum temperature of a particular month.

After acclimatization of crab *Scylla serrata* in the laboratory conditions for 2 days, the rates of excretion in the form of ammonia, urea, free amino acid, trimethyl amine oxide (TMAO) and aquatic respiration of each crab were measured. Rates of ammonia excretion by using phenol reagent, urea excretion by using diacetyl monoxime reagent, and amino acids (small peptides) excretion by using ninhydrin reagent were estimated[10]. Rate of trimethyl amine oxide excretion was also estimated by using Fe - EDTA reagent[11]. Rate of aquatic respiration was measured by measuring the dissolved oxygen in the water[12].

Various tissues namely muscles, gills, hepatopancreas and heamolymph were collected to estimate various biochemical parameters. Heamolymph was collected with the help of syringe from the last pleopod of the crab. Tissues from individual crabs were homogenized separately in ice cold distilled water. A part of the homogenate was deproteinized and used for estimation of total carbohydrates and free sugars. From other part of homogenate free amino acid, triglycerides, free fatty acids and protein was extracted using different solvents, acid and alkali[13]. Total carbohydrates by using anthrone reagent[14], free sugars by using areseno molybdate reagent[15], free amino acids by using ninhydrin reagent[16], total proteins by folin- phenol reagent[17], triglycerides by using chromatotropic acid[18] and free fatty acids by using Sodium dithiocarbamate colour reagent[19] were quantified in different tissues.

Experimental data was represented in the form of Mean $\pm$ SE of six individual crabs. For comparison between Full moon and New moon data of a particular season, student '*t*' test was performed by using Microsoft excel 2007 sheet. "*F*" test (ANOVA) was also performed for understanding the seasonal variation. This was done by downloading the software from http-//www.statpages.org. Criterion of significance level for student '*t*' test as well as "*F*" test adopted was 5%.

RESULTS AND DISCUSSION

Mud crab, *Scylla serrata* commonly inhabit in sheltered estuaries, mud plates, mangrove forests. They are exposed to large number of environmental variables including annual and daily tidal cycles, lunar cycles etc. Adaptation of an organism to a particular environment involves an integrated response to changes of environmental parameters depending on its homeostatic control mechanism. Such integrated response may be exerted at different biochemical, physiological and behavioural level. In this context, the effect of single variable is largely overridden by the positive or negative synergism between various variable of given environment[20].

The levels of protein, free amino acids, total carbohydrates, and free sugars, total fat and free fatty acids are expression of animal's adaptive characters and its strategies for adaptation in a particular habitat. Many biotic factors (such as breeding cycle of the animal, availability of the food) and abiotic factors (like salinity, photoperiod, tidal cycles etc.) can strongly affect the biochemistry and physiology of crustacean[21-23]. Increase in the concentration of total protein maximum by 5% (figure 3), free amino acid by 5-25% (figure 4), total carbohydrates by 10-25% (figure 5), free sugar by 10-35% (figure 6), total fat by 5-25% (figure 7), free fatty acid by 5-20% (figure 8) in heamolymph, hepatopancreas, gills and muscles of *Scylla serrata* on full moon day over the new moon day throughout the year is associated with the watery phase of the crab as observed on new moon day. Accumulations of various biomolecules in various tissues indicate the augmentation of biosynthetic machineries or due to maximum availability of the food on full moon day. In the present result we also recorded the 20 -60% higher level of aquatic oxygen consumption (figure 1) and 10-30% increased rate of ammonia , urea and amino acids excretion in *Scylla serrata* (figure 2) on full moon day as compared the same on new moon day during an annual cycle. This indicates the higher activity of the mud crab on full moon day.

The crab migrates to the shore / mangrove vegetation for feeding after the 1st quarter of lunar cycle and remains very active till the 3rd quarter with a peak on full moon day. That might be the reason for higher catch of the crab on full moon day[5]. These changes in the biochemistry and the physiological function in crab might also be associated with the moulting behaviour of crabs and tidal cycles of the oceans. Several reports are available which specify the direct correlation between moulting behaviour of crab and tidal rhythms as reviewed by[24,25]. Accumulation of water in the body or in various tissues at post-moult and reduction of the same at premolt is achieved by controlling the osmoregulatory as well as excretory processes[26]. Perhaps that is the reason for accumulation of various biomolecules in the tissues with increased rate of excretion and aquatic respiration on full moon day or during the 2nd quarter of lunar cycle. About 15% increased rate of trimethyl amine oxide excretion during full moon over the new moon period were observed only in monsoon and post monsoon season only. Why the increased rate of trimethyl amine oxide excretion is noticed only during particular season can't be explained in present circumstances.

A significant seasonal variation in the rate of aquatic respiration (F=5.2-6.6, P< 0.01), rates of excretion of ammonia (F=6.7-3.7, P<0.05), urea (F=14.5-13.01, P<0.01), free amino acids (F=42.4-81.02, P<0.01) and trimethyl amine oxide (F=6.4-6.6, P<0.01) were noticed in *Scylla serrata* irrespective of the phases of moon. Besides, significant seasonal variation in the biochemical compositions (total protein, free amino acids, total carbohydrates, free sugars, triglycerides and free fatty acids) in various tissues (muscles, gills, hepatopancreas and heamolymph) of mud crab were also recorded. 2.5 - 8% higher accumulation of free amino acid (Fig. 4) and 3 - 8.5% reduction in the tissue total protein (figure 3) in gill, hepatopancreas and heamolymph of mud crab during winter over the same of summer months indicate the utilization of more protein during winter in order

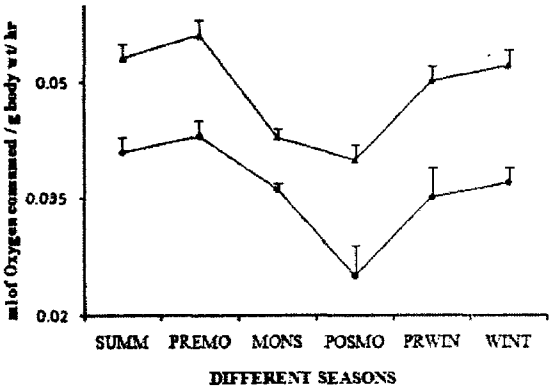

Figure 1: Seasonal variation in the rate of aquatic respiration (ml of oxygen consumed/g body wt / hr) of mud crab, *Scylla serrata* during new moon and full moon phases of lunar cycle.

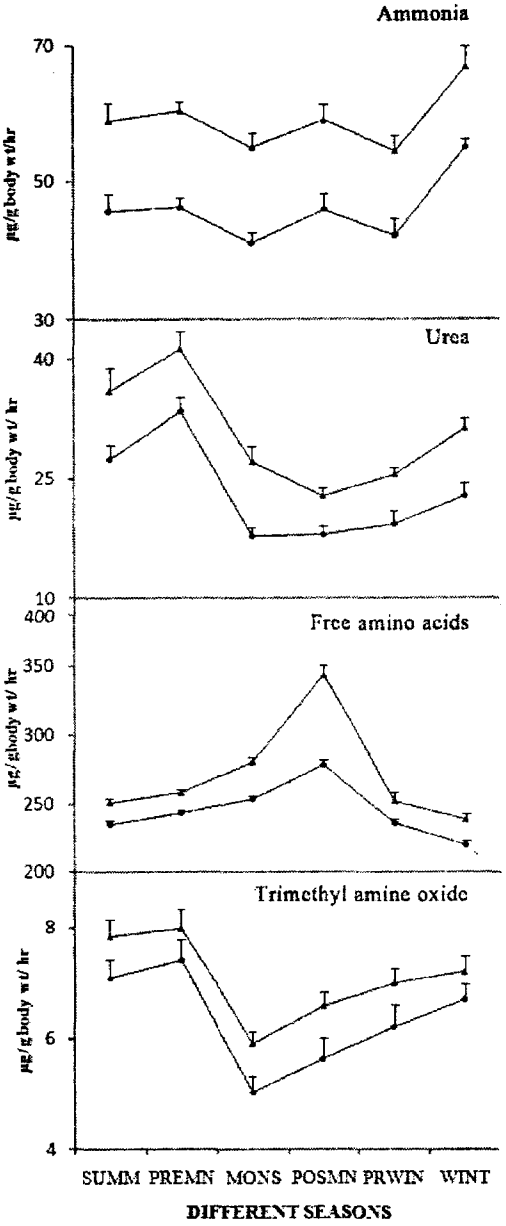

Figure 2: Seasonal variation in the rates of excretion (µg / g body wt / hr) of mud crab, *Scylla serrata* during new moon and full moon phases of lunar cycle.

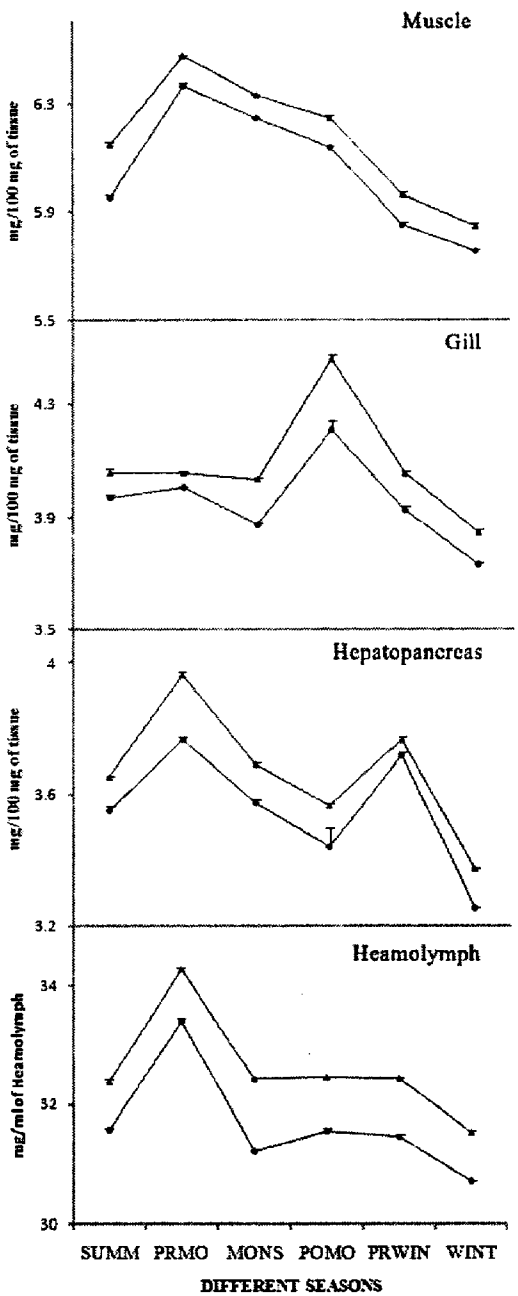

Figure 3: Seasonal variation in concentration of total protein in muscles, gills, hepatopancreas (mg / 100 mg of tissue) and heamolymph (mg / ml) of mud crab, *Scylla serrata* during new moon and full moon phases of lunar cycle.

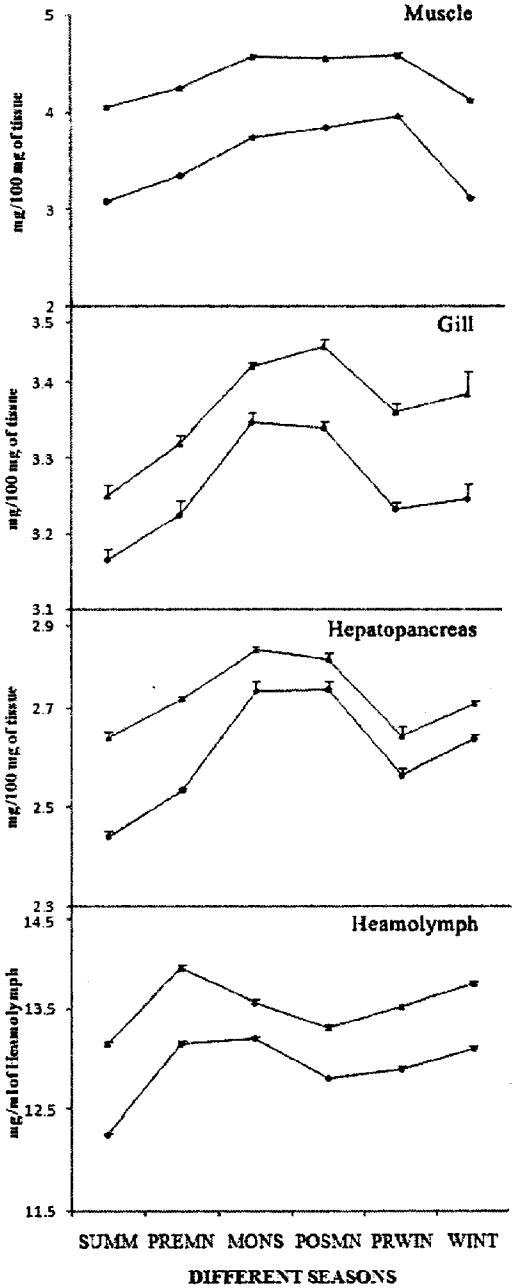

Figure 4: Seasonal variation in concentration of free amino acids in muscles, gills, hepatopancreas (mg / 100 mg of tissue) and heamolymph (mg / ml) of mud crab, *Scylla serrata* during new moon and full moon phases of lunar cycle.

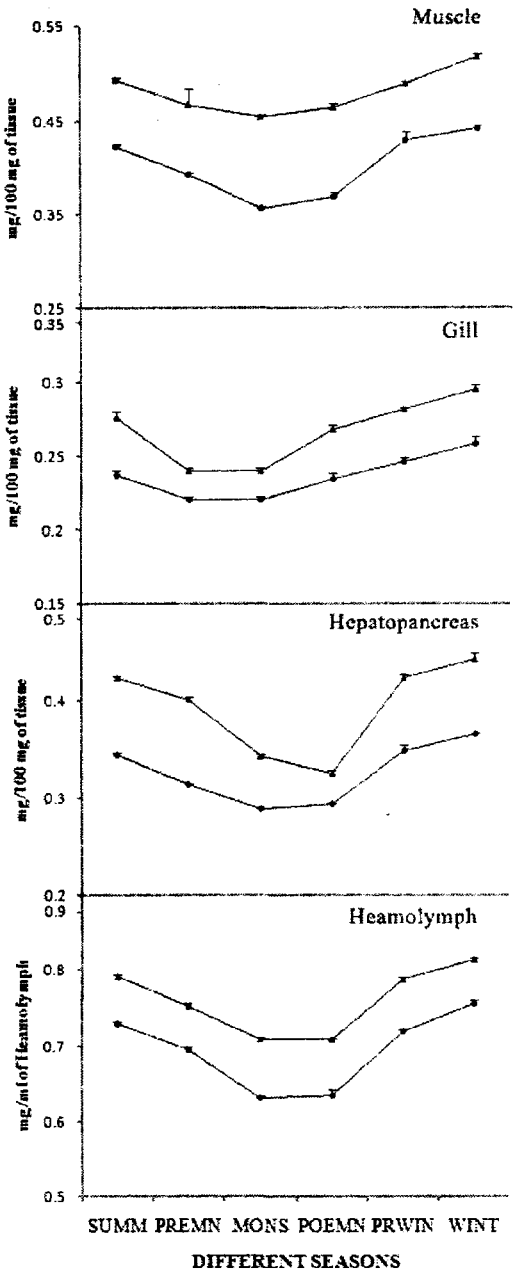

Figure 5: Seasonal variation in concentration of total carbohydrates in muscles, gills, hepatopancreas (mg / 100 mg of tissue) and heamolymph (mg / ml) of mud crab, *Scylla serrata* during new moon and full moon phases of lunar cycle.

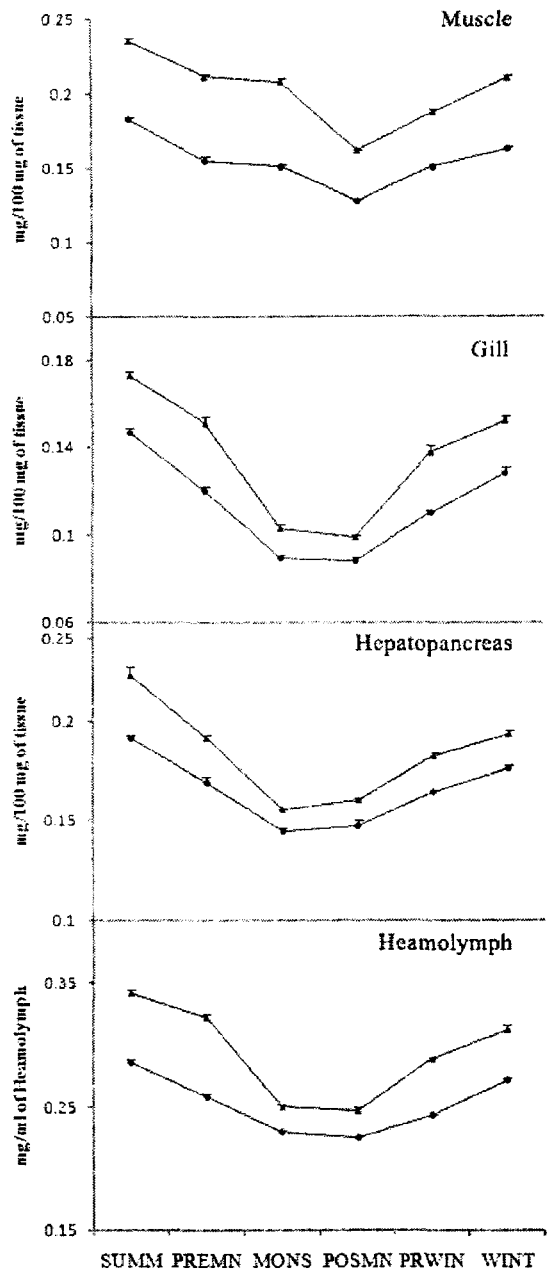

Figure 6: Seasonal variation in concentration of free sugars in muscles, gills, hepatopancreas (mg / 100 mg of tissue) and heamolymph (mg / ml) of mud crab, *Scylla serrata* during new moon and full moon phases of lunar cycle.

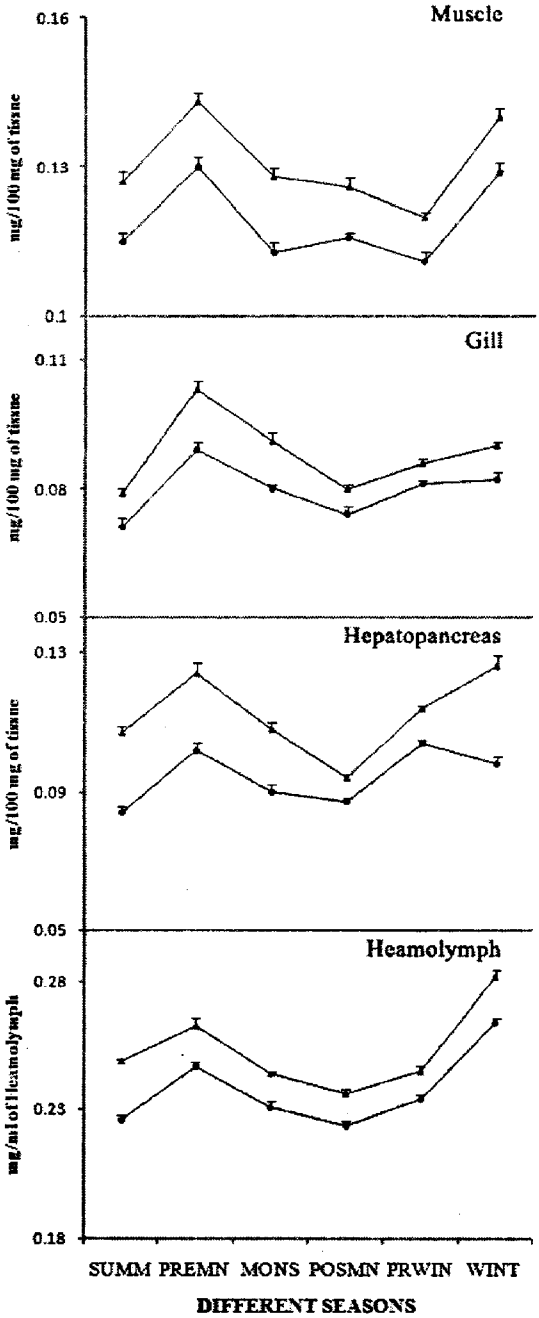

Figure 7: Seasonal variation in concentration of triglycerides in muscles, gills, hepatopancreas (mg / 100 mg of tissue) and heamolymph (mg / ml) of mud crab, *Scylla serrata* during new moon and full moon phases of lunar cycle.

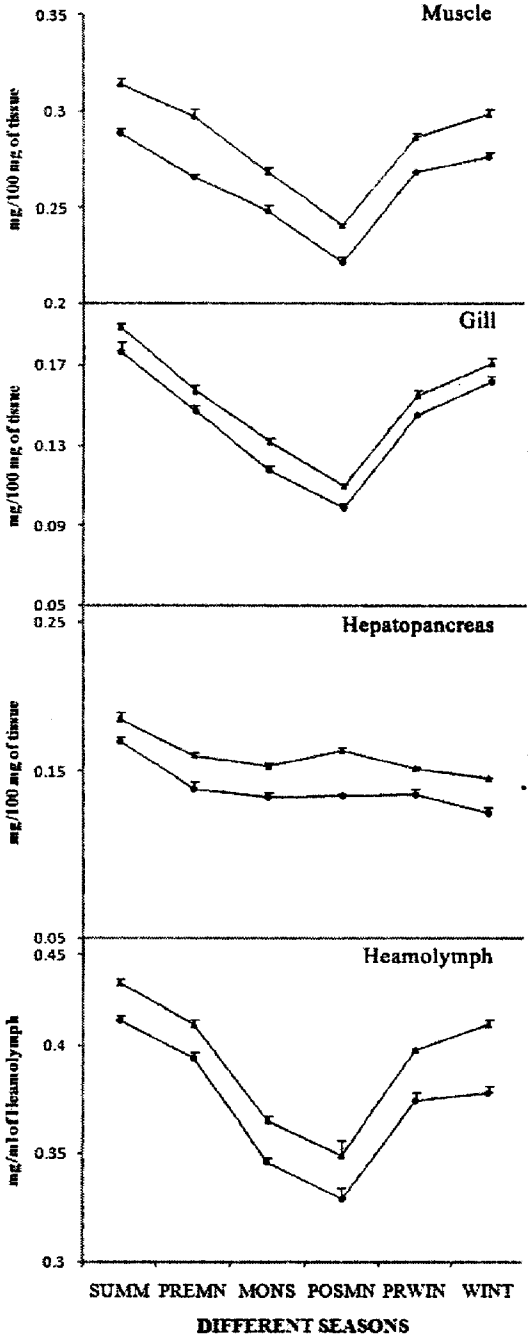

Figure 8: Seasonal variation in concentration of free fatty acids in muscles, gills, hepatopancreas (mg/100 mg of tissue) and heamolymph (mg/ ml) of mud crab, *Scylla serrata* during new moon and full moon phases of lunar cycle.

to meet the energy demand of the body. It has been reported by[27] that at low temperature the juvenile *Scylla serrata* enhanced their catabolic break down of protein to produce more ammonia which were essential to maintain the osmolarity. Our results also indicate the higher rate protein catabolism during winter than the summer. 5 – 17% lower rates of urea, free amino acid and trimethyl amine oxide excretion along with 13 – 20% higher rates of ammonia excretion during winter over the same during summer months, clearly indicates a shift towards ammonoteleism during winter months. However, further studies are essential to clarify the activity of enzymes involved in nitrogen catabolisms and the role of electrolytes in seasonal adaptation. Maximum rise in the rate of ammonia, urea excretion as well as TMAO excretion (figure 2) along with elevated rate of aquatic respiration (figure 1) during pre monsoon period clearly indicates the preparation of gametogenesis of mud crab. Higher rates of oxygen consumption along with nitrogen excretion as an indicator of the endogenic condition leading to gametogenesis of aquatic organism[28]. The mud crab *Scylla serrata* spawn during the monsoon or post monsoon in the coastal states of India[29].

The observed maximum depletion in the levels of total carbohydrates (figure 5) as well as in the level of free sugars (figure 6) and total protein (figure 3) along with increased level of free amino acid (figure 4) during monsoon or post monsoon period clearly indicates the utilization of carbohydrates and protein to gain the required energy for the breeding activity of crab. Lipids play a vital role in the biological activities of crustacean. Besides, providing energy, they maintain the cellular and physiological integrity of the organism. The quantity and quality of lipid moieties through the life cycle of crustacean play significant role[30]. The lipid reserves and its mobilization also very important during the breeding activity of crustacean[31]. A high energy yielding metabolism is very essential for the spawning and reproduction of the organism. For the reproduction, which includes gametogenesis, vitellogenesis as well as spawning, lipid reserves utilization and mobilization of these reserves is very essential. This observation was in accordance with the early reports of[3,4,32]. During our study we have recorded the maximum depletion of triglyceride (figure 7) during the breeding period of Scylla serrata. The challenge in supply of maximum energy for the breeding activity can be met from mobilization of fat more rather than protein or carbohydrate.

In conclusion, the result presented in the paper suggest that in *Scylla serrata* the environmental conditions (temperature, rain fall, lunar phase) and the reproductive activity appear to be main processes influencing the metabolism and the seasonal pattern in biochemical composition. The observed differences in physiological and biochemical parameters during new moon and full moon phases of the lunar cycle may be associated with the moulting behaviour or the feeding activity of the mud crab, *Scylla serrata.*

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