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Variations in gross biochemical composition in relation to the gametogenic cycle of the baby clam, *Marcia opima* (Gmelin), from two geographically separated areas

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ABSTRACT

Gross biochemical composition and condition index were investigated in two geographically separated populations of the baby clam, *Marcia opima* during December 1998 - January 2000. Proteins, lipids, total carbohydrates and glycogen showed similar patterns of the variation according to the reproductive cycles in clams from both the areas, highlighting the importance of the reproductive cycle and physiological features of the species and also the influence of environmental conditions in determining biochemical composition. Proteins and lipids were maximum in the mature clams and decreased during and after spawning. Lipids were higher in females. Seasonal variations of lipid were inversely related to glycogen contents. Total carbohydrates and glycogen increased during summer and decreased during monsoon. Condition index was influenced by environmental conditions, which further determined the spawning season.

Keywords: Biochemical composition, Clam, Condition index, Gametogenic cycle, *Marcia opima*

Introduction

The venerid clam *Marcia opima* is a commercially important and largely exploited species. According to Narasimham (2005), the estimated annual production of the species is 500 t and is exploited from several estuarine areas of peninsular India. The meat is consumed in domestic markets, while the shells are used by lime, shell grit, cement, and calcium carbide industries. It is a major candidate species for large-scale aquaculture. In order to improve the methods for cultivating *M. opima*, a detailed fundamental knowledge on the reproductive cycle, spawning periods and the influence of biochemical constituents on the reproductive cycle is essential. In addition, information on the biochemical constituents of the meat would help to identify the best harvest season for the species coinciding with high nutritive value. The baby clam, *M. opima*, has so far been indicated as *Katelysia opima* in Indian waters (John Taylor, personal communication). Larval rearing and large scale spat production of this species has been successfully achieved for the first time in India by Muthiah *et al.* (2002). The reproductive biology of the clam from south-east and south-west coasts of India was studied in detail by Suja and Muthiah (2007).

Studies of energy metabolism are concerned with the ways in which major carbohydrate; lipid and protein fuels are used by an organism to produce energy. As the

nutritional and energy demands of marine animals are not constant, and are affected by exogenous factors such as food availability and temperature, as well as by endogenous factors such as energy demands for reproduction, metabolic reserves accumulated in tissues may be used in energy production or converted into various biochemical components (Berthelin *et al.*, 2000). Marine bivalves show seasonal cycles of energy storage and utilization that are closely related to reproductive activity (Giese *et al.*, 1967). Masumoto *et al.* (1934) reported that environmental factors apparently play a dominant role in determining events in the storage cycle. Gabbot (1975) stated that seasonal metabolic activities in molluscs result from complex interactions among food availability, environmental conditions, growth and reproduction.

Several studies on biochemical cycles in bivalves have been carried out in relation to reproduction (Jayabal, 1994; Berthelin *et al.*, 2000). The general observations have been that the amount of carbohydrate, proteins and lipids increase as gonad development proceeds and then declines following spawning (Beninger and Stephen, 1985; Robert *et al.*, 1993).

The present study, aimed to compare the seasonal variations of the gross biochemical composition in *M. opima* populations from Tuticorin Bay, on the south-east coast (8° 45' N and 78° 12'E) and Ashtamudi Estuary on the south-west coast (9° 28' and 76° 28'E) of India.

Materials and methods

Sampling

Clams were collected at monthly intervals from Tuticorin Bay between December 1998 and January 2000. From Ashtamudi Estuary, sampling was done in alternate months during March 1999 to January 2000. A wooden frame of 50 m² was placed in the exposed area of the intertidal zones of the sampling sites during low tide and the clams were hand picked for the study.

Temperature and salinity of seawater were measured during sampling. The temperature measurements were taken at 0.5 to 1.0 m depth in the water column. Salinity of water samples collected from the sampling sites was estimated by Mohr-Kundson titration method as given by Strickland and Parsons (1972). After sampling, clams were transported to the laboratory and placed in filtered seawater for 3-4 h in order to purge their pseudofaeces and stomach contents.

Gametogenic activity

Twenty clams from Tuticorin and ten clams from Ashtamudi were segregated for histological examination during the sampled months. The different gametogenic stages were then identified in the stained gonad sections as maturing, mature, partially spent, spent and indeterminate. The stages described were according to a modified version of that done by Nagabhushanam and Mane (1975). When two or more stages occurred simultaneously in a single section, classification of the stage was based up on the condition of the majority of follicles present in the section. A detailed study on the histology of gonad maturity stages of baby clam has been conducted by Suja and Muthiah (2007).

For determination of condition factor, thirty samples were opened and soft tissues shucked out. The tissues as well as shells were marked, dried at 60 °C for 48 h and then weighed to obtain the dry weights of shell and flesh. The condition index was calculated according to Walne (1976) as:

$$\text{Condition index} = \frac{\text{Dry flesh weight (g)}}{\text{Dry shell weight (g)}} \times 100$$

Biochemical determinations

For biochemical determinations, clams were randomly selected and kept in freezer at -20 °C. Before biochemical estimation, gonad smears were taken to assess the maturity stages. Depending on gamete maturation, clams from both the sampling areas were segregated according to sex as two pools each of males and females in March, May, July, September, November 1999 and January 2000. The frozen

samples were analyzed after lyophilization. The determination of the biochemical components was carried out in triplicate. Protein content was determined following the method of Lowry *et al.* (1951), carbohydrate and glycogen contents according to Dubois *et al.* (1956). Determination of lipids was done by the sulphophosphovanillin method of Barnes and Blackstock (1973). The results were expressed as microgram per milligram dry weight ($\mu\text{g mg dry weight}^{-1}$).

Statistical analyses

The Pearson product-moment correlation was used to examine correlation between the male and female reproductive cycles and condition index. An unpaired t-test was used to ascertain differences between salinities and condition indices at the two study sites, and the Mann-Whitney U-test to ascertain differences in temperatures. All comparisons of the biochemical compositions between the sampling sites were made using ANOVA.

Results and discussion

The sampled baby clams ranged from 26.2 mm to 57.6 mm length in antero-posterior axis. The mean length of monthly sampled clams from Tuticorin Bay varied between 27.59 mm and 36.77 mm, while that of Ashtamudi Estuary was between 32.12 mm and 37.11 mm.

Seasonal variations in temperature and salinity from the study sites are given in Fig.1a and 1b. There was no significant difference between temperatures at the two sites (Mann-Whitney U test, $p=0.724$), whilst Tuticorin Bay experienced significantly higher salinities than Ashtamudi Estuary (unpaired t-test, $p<0.01$).

Fig. 2a to 2d represents the percentage of male and female clams in various reproductive stages at Tuticorin Bay and Ashtamudi Estuary respectively. The baby clams

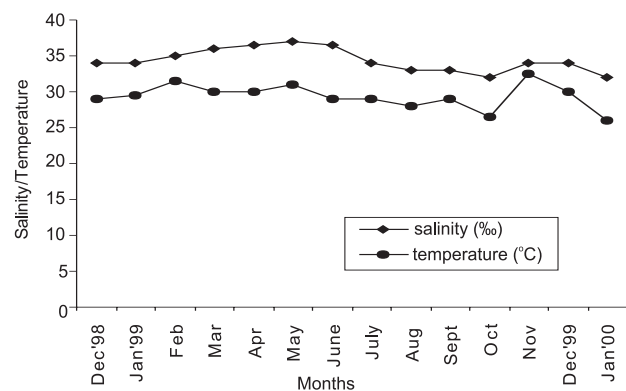


Fig. 1(a). Seasonal variation in sea temperature and salinity at Tuticorin Bay sampling site from December 1998 to January 2000.

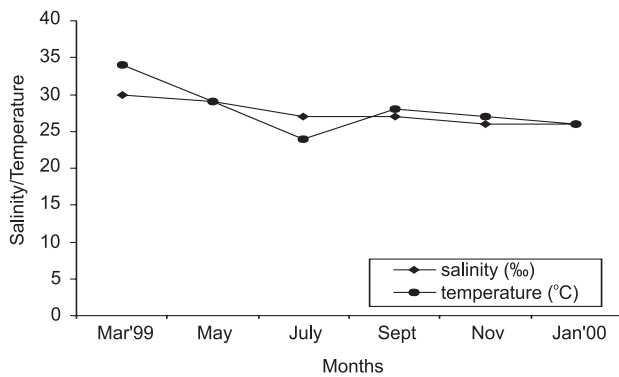


Fig. 1(b). Seasonal variation in sea temperature and salinity at Ashtamudi Estuary sampling site from March 1999 to January 2000.

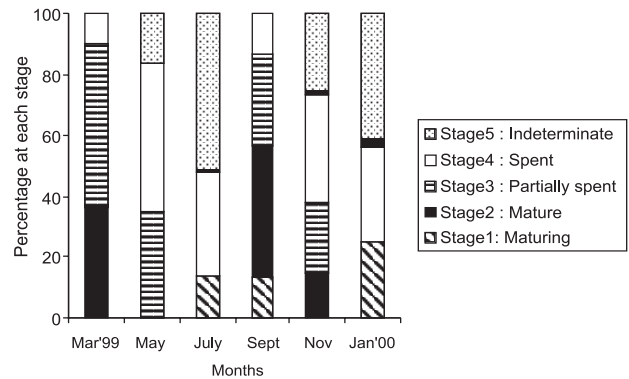


Fig. 2(c). The percentage of male baby clams in various reproductive stages at Ashtamudi Estuary from March 1999 to January 2000.

from Tuticorin Bay showed onset of gametogenesis in January. They spawned continuously from May to July. A second cycle of gametogenic activity resumed in July, followed by a spawning season from September to December.

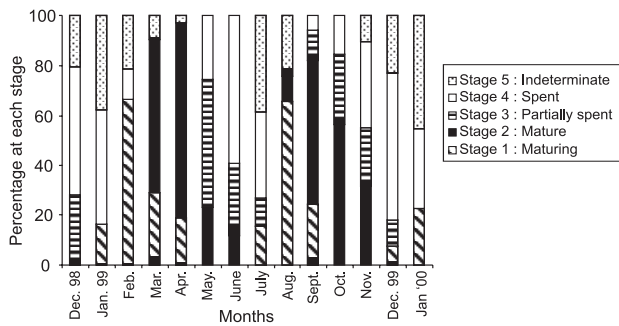


Fig. 2(a). The percentage of male baby clams in various reproductive stages at Tuticorin Bay from December 1998 to January 2000.

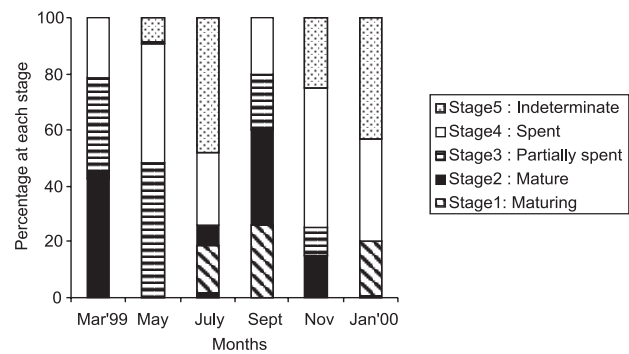


Fig. 2(d). The percentage of female baby clams in various reproductive stages at Ashtamudi Estuary from March 1999 to January 2000.

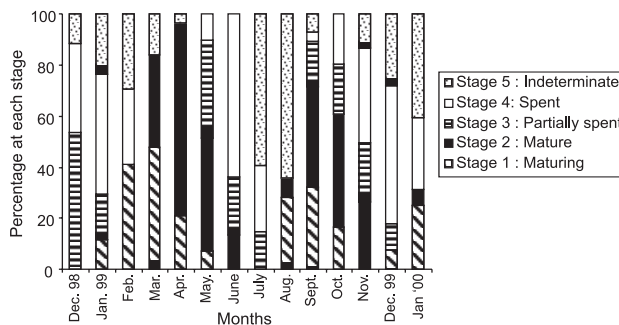


Fig. 2(b). The percentage of female baby clams in various reproductive stages at Tuticorin Bay from December 1998 to January 2000.

In Ashtamudi Estuary, the first spawning period was from March to May and the second spawning season was from September to December. There was a significant positive correlation (Pearson product-moment correlation, $r = 0.756$, $p < 0.01$) between the male and female gametogenic cycles of clams of Tuticorin Bay and of Ashtamudi Estuary ($r = 0.734$, $p > 0.05$).

No significant correlation was observed between male gametogenic cycle with respect to temperature (Pearson product - moment correlation $r = -0.365$, $p > 0.05$) or salinity (Pearson product- moment correlation $r = 0.125$, $p > 0.05$).

The condition factor of bivalves can act as indicator of reproductive activity and the condition of clam is dependent on the gametogenic activity. The condition index showed higher values in Tuticorin Bay clams (Fig. 3). The individual values of average condition index during the sampled period varied from 6.50 in November 2000 to 9.36 in April 1999 with an average of 7.93. The clams of Ashtamudi Estuary showed minor variations in condition

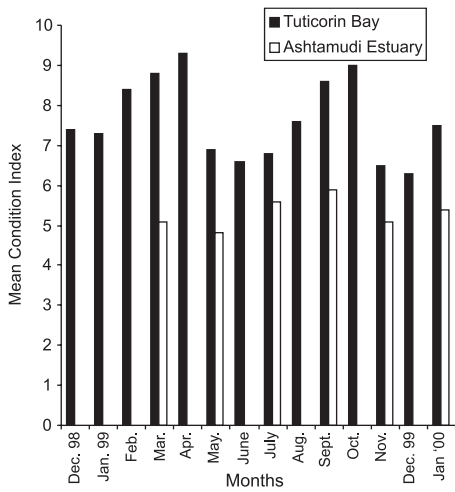


Fig. 3. Seasonal changes in condition index of baby clams in Tuticorin Bay from December 1998 to January 2000 and in Ashtamudi Estuary during the sampled months from March 1999 to January 2000.

index with a maximum value of 5.9 in September 1999 and a minimum value in May 1999 (4.8). Although the index appears to be higher for clams in Tuticorin Bay in comparison to Ashtamudi Estuary, there was no significant difference between condition indices for corresponding months at both sites (unpaired t- test, $p=0.0018$). The male gametogenic cycle significantly correlated with the condition index in both the sampling areas (Pearson product - moment correlation, $r=0.737$ for Tuticorin Bay and $r=0.899$ for Ashtamudi Estuary, $p>0.05$). A significant correlation was observed between the female gametogenic cycle and the condition index (Pearson product - moment correlation, $r=0.786$, $r=0.758$ for Tuticorin Bay and Ashtamudi Estuary respectively; $p>0.05$).

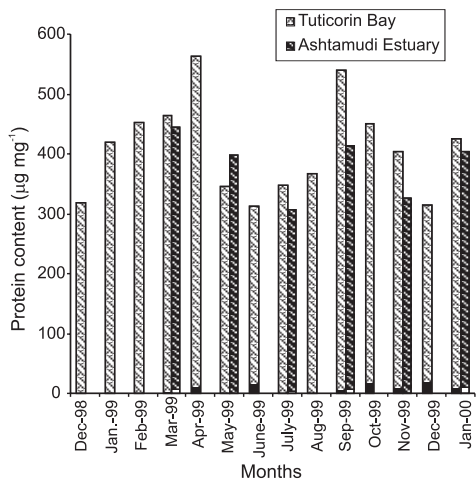


Fig. 4(a). Mean monthly values of protein in baby clams collected at Tuticorin Bay and Ashtamudi Estuary.

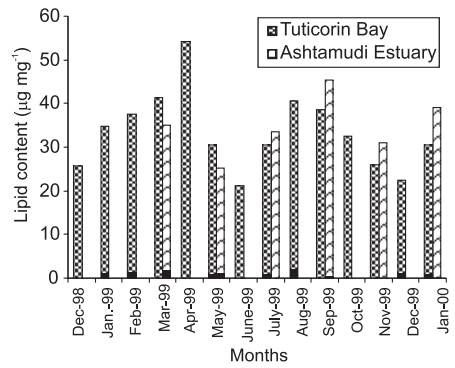


Fig. 4(b). Mean monthly values of lipids in baby clams collected at Tuticorin Bay and Ashtamudi Estuary.

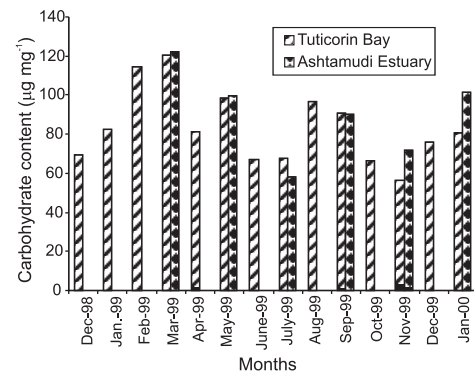


Fig. 4(c). Mean monthly values of carbohydrates in baby clams collected at Tuticorin Bay and Ashtamudi Estuary.

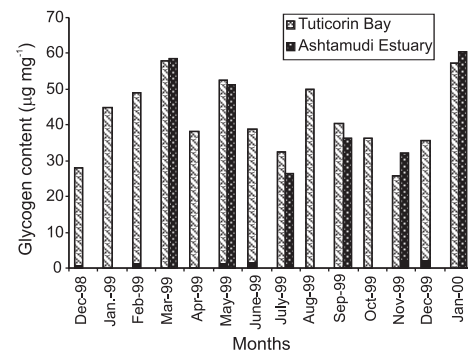


Fig. 4(d). Mean monthly values of glycogen in baby clams collected at Tuticorin Bay and Ashtamudi Estuary.

The changes in biochemical composition of baby clams were almost similar in both the sampling areas (Fig. 4a - 4d). The highest value of protein was detected in clams of Tuticorin Bay in April 1999 ($563.2 \mu\text{g mg}^{-1}$) followed by September 1999 ($540.5 \mu\text{g mg}^{-1}$). The clams of Ashtamudi Estuary showed the highest protein value in March 1999 ($444.0 \mu\text{g mg}^{-1}$) and September 1999 ($414.0 \mu\text{g mg}^{-1}$). The lowest values were recorded in June

1999 (311.8 $\mu\text{g mg}^{-1}$) and in July 1999 (307.5 $\mu\text{g mg}^{-1}$) in samples of Tuticorin Bay and Ashtamudi Estuary respectively.

In clams collected from Tuticorin Bay, lipid content was highest during April 1999 (54.2 $\mu\text{g mg}^{-1}$). The highest lipid value was observed in clams of Ashtamudi Estuary in September 1999 (45.4 $\mu\text{g mg}^{-1}$). Similar to proteins, the lowest lipid values were reported in June 1999 (21.3 $\mu\text{g mg}^{-1}$), in the clams of Tuticorin Bay and in May 1999 (25.1 $\mu\text{g mg}^{-1}$), in the clams of Ashtamudi Estuary. The carbohydrate values of clams collected from Tuticorin Bay showed an increasing trend from December 1998 to March 1999 when the value reached the highest (120.4 $\mu\text{g mg}^{-1}$). The value decreased in April when majority of the clams were ripe. An increase in the carbohydrate content was observed in May 1999 (98.2 $\mu\text{g mg}^{-1}$). A sudden fall in the carbohydrate values was recorded during June 1999 and July 1999 and there was a rise in the values in August 1999 and September 1999. For the clams of Ashtamudi Estuary, the highest value of carbohydrates was observed in March 1999 (122.4 $\mu\text{g mg}^{-1}$) and the lowest value in July 1999 (58.3 $\mu\text{g mg}^{-1}$). Similar to the carbohydrate values, the clams of Tuticorin Bay showed maximum values of glycogen in March 1999 (57.9 $\mu\text{g mg}^{-1}$). The values were minimum in November 1999 (25.7 $\mu\text{g mg}^{-1}$). Glycogen patterns of clams of Ashtamudi Estuary varied with maximum values obtained in March 1999 (58.4 $\mu\text{g mg}^{-1}$) and minimum values in July 1999 (26.4 $\mu\text{g mg}^{-1}$).

Comparing clams from Tuticorin Bay and Ashtamudi Estuary, the protein values were significantly higher at Tuticorin Bay from September 1999 to November 1999 ($p < 0.001$ in September and $p < 0.01$ in November). The lipid values of clams at Ashtamudi Estuary were significantly higher in September 1999 ($p < 0.01$) and November 1999 ($p < 0.05$). The carbohydrate showed higher values at Tuticorin Bay in July 1999, being significantly different ($p < 0.01$) with significantly higher values at Ashtamudi Estuary from November 1999 to January 2000 ($p < 0.05$). The glycogen values were significantly different from July 1999 to November 1999 ($p < 0.01$ in July, $p < 0.01$ in September and $p < 0.05$ in November).

Comparing the biochemical compositions in male and female clams, protein contents were irregularly variable, without clear trends. Lipids were always higher in female clams from both the sampling areas. Carbohydrate values were higher for females at Tuticorin Bay from March 1999 to November 1999, whereas the females at Ashtamudi Estuary showed higher values only in March 1999. Glycogen was higher for female clams at Tuticorin Bay except for September 1999 (Table 1).

The fluctuations in the biochemical constituents studied may be interrelated to maturation of gonads and availability of food. This also highlights the predominant role played by the reproductive and physiological characteristics of the species, *M. opima* over and above the differences in environmental conditions of the two sampling

Table 1. Comparisons between mean protein, lipid, carbohydrate and glycogen values in male (M) and female (F) clams collected from Tuticorin Bay (T) and Ashtamudi Estuary (A) ($\mu\text{g mg}^{-1}$)

	Sex	March 1999	May 1999	July 1999	September 1999	November 1999	January 2000
Proteins							
T	M	452.3	376.2	366.8	520.3	426.1	414.5
	F	476.2	314.3	328.2	560.6	380	436.6
A	M	424.5	411.4	302.2	406.9	316.8	386.4
	F	463.5	386.4	312.8	421.1	335.2	422.5
Lipids							
T	M	38.2	28.7	31.6	37.9	22.5	26.6
	F	44.6	32.5	29.5	39.3	29.5	34.2
A	M	31.8	25.6	32.6	47.2	29.4	34
	F	38.2	24.5	34.6	53.6	32.7	44.5
Carbohydrates							
T	M	96.8	126.5	58.7	86.6	54.6	82.5
	F	99.5	114.3	76.5	94.5	58.2	78.2
A	M	94.9	124.2	60.4	97.3	74.3	104.8
	F	104.2	120.6	56.2	83.4	69.5	97.7
Glycogen							
T	M	51.1	50.3	31.5	43.6	25.5	54.8
	F	63.8	54.5	33.7	37.2	25.9	59.5
A	M	61.7	52.5	28.6	32.9	33.3	63.2
	F	55.1	49.7	24.2	39.7	30.8	57.4

areas. Compared to other biochemical constituents, the protein values remained high throughout the year. As observed in the present study, an increase in the protein content during maturity and a decrease during spawning has been reported earlier in many other bivalves, such as *Villorita cyprinoides* and *Meretrix casta* (Lakshmanan and Nambisan, 1979), *Meretrix meretrix* (Jayabal and Kalyani, 1987), *Katelysia opima* (Jayabal, 1994), and *Crassostrea gigas* (Berthelin *et al.*, 2000).

The seasonal variations in lipids showed a similar pattern like that of protein. The lowest lipid values observed in the clams of Tuticorin Bay and Ashtamudi Estuary coincided with respective spawning seasons. A similar condition has been reported in *Crassostrea gryphoides* by Durve and Bal (1961) and in *Katelysia marmorata* by Joshi and Bal (1965). A transfer of lipids from somatic to gonadal tissue may occur, as suggested by the inverse relationship between somatic and gonadal lipid levels observed in *Chlamys opercularis* (Taylor and Venn, 1979). Maximum lipid values for the clams from both the areas appeared to be associated with the gonad development. Generally, values of lipids in female were slightly higher than male as reported by Jayabal and Kalyani (1987) in the hard clam, *M. meretrix* and Jayabal (1994) in the estuarine clam *K. opima*. Beninger and Stephen (1985) observed that lipids are the main component of oocytes.

Carbohydrate patterns are strongly related to those of glycogen. Glycogen is the main energy reserve in adult bivalves, being used during gametogenesis and in conditions of nutritional stress. Several workers have reported carbohydrate and glycogen maxima in bivalves immediately preceding and during gamete maturation (Ansell, 1972; Shafee, 1978). In the present study, an increase in the carbohydrate and glycogen values was observed during active gametogenesis in clams of both the sampling areas. The lowest values of carbohydrate and glycogen were reported when majority of the clams were mature and also during the monsoon period.

High values of carbohydrate and glycogen were reported in bivalves when they are sexually inactive (Joshi and Bal, 1965; Nagabhushanam and Deshmukh, 1974). Glycogen has long been considered to be the principal energy reserve of adult marine bivalves (Giese, 1969) especially under conditions of nutrient stress (Barber and Blake, 1985). Giese *et al.* (1967) showed that gonads of *Tivela* had least carbohydrate storage when mature gametes were present, suggesting a massive conversion of carbohydrate into gamete tissue. In bivalves, gonad development may involve the metabolic conversion of glycogen to lipid (Gabbott, 1975). Minimum values of glycogen content coincided with maximum values of lipid contents, probably due to the conversion of the former component into the latter.

The increase in glycogen and total carbohydrate content appears to be related to periods of maximum phytoplankton abundance in water, whereas the minima are found when primary production decreases (Lakshmanan and Nambisan, 1979). A sudden increase in the carbohydrate and glycogen values at Tuticorin Bay and Ashtamudi Estuary in May 1999 may be related to the increased phytoplankton production during summer. Low values of carbohydrate and glycogen content during and after the monsoon period may be due to the non-availability of sufficient phytoplankton, which is associated with low salinity (Jayabal, 1994). A decrease in glycogen and carbohydrate values from May onwards due to the increase in water content has earlier been reported in *M. meretrix* (Nagabhushanam and Deshmukh, 1974) and in *K. marmorata* (Joshi and Bal, 1965) from Indian waters.

Studies on the same genus from Indian waters suggests that the biochemical constituents in *K. opima* from Vellar Estuary increased during maturation of gonads and decreased during active spawning and monsoon months due to heavy inflow of freshwater in to the estuary, which further lowered the phytoplankton production ((Jayabal, 1994). An increase in the values of biochemical components during the period of active gametogenesis and a fall in the values during spawning and monsoon season were also reported in *K. marmorata* from Mahim Bay by Joshi and Bal (1965). Hence the fluctuations in the biochemical constituents observed may be interrelated to maturation of gonads and availability of food. Present work also reveals that the biochemical changes in the estuarine clam, *M. opima* are mainly influenced by reproductive cycle and also by food availability. The result is further confirmed by the condition index values, which exhibited a similar pattern in the same period in respective sites.

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