8. BIOCHEMICAL COMPOSITION

8.1. INTRODUCTION

Bivalve mollusks like Oysters, Mussels, Clams scallops etc are highly esteemed delicious sea foods and are considered as next in importance only to fishes and prawns. Much information is available on the biochemical constituents of various bivalves and the earliest work on this aspect was made by John (1814) and Orton (1924) on Ostrea edulis by Atwater (1892), Hunter and Harrison (1928) and Nilson and Coulson (1939) on Crassostrea madrasensis; by Clements and Hutchinson (1939) on Mytilus edulis by Etroma (1928) on Anadara granosa and by Meigs (1915) on Venus mercenaria. Giease (1969) reviewing the biochemical composition of different body parts of the bivalves. Ansell (1974a) studied the seasonal changes in the biochemical composition and the tissue weight of the Pectinid bivalves. Seasonal variations in biochemical constituents were reported by Ansell and Lander (1967) in Venus mercenaria; by Ansell and Trevallion (1967) in Tellina tenuis; by Giese et al (1967) in Mytilus edulis by Ansell (1972) in Donax vitatus by Ansell et al (1973) in Donax incarnatus and Comely (1974) in Pecten maximus. Similar observation have been made on Chlamys septemradiata (Ansell, 1974b; Taylor and Venn, 1979) and Mytilus edulis (Zurberg et al., 1979).

In India, Studies on biochemical composition of bivalves were there of Venkataraman and Chari (1951) on Meretrix casta and Crassostrea madrasensis; Nagabhushanam (1961) on Martesia striata; Rahaman (1965,66) on Meretrix casta and Sanguriolaria diphos; George John (1980) on Anadara rhombea; Jayabal (1984) on Meretrix meretrix and Balasubramaniyan (1984) on Meretrix casta; Krishnakumari et al. (1977) in Meretrix casta, Durve and Bal 1961; Joshi and Bal 1965; Kasinathan 1964a, Band 1967; Saraswathi and Nair 1969; Wafer 1974; George and Nair 1975; Salih 1979; Jayabal and Kalyani (1986) In western waters biochemical studies have been carried out by Park et al(2001) in ark shell Scapharca broughtonii. Many studies
have related changes in the biochemical composition of bivalves with the reproductive
cycle mostly in the natural environment. Energy storage and biochemical cycle are
closely related to reproductive activity in marine bivalves (Giese, 1969; Bayne, 1976;
Ojea et al., 2004). Beninger and Lucas (1984) and Bressan and Marin (1985) studied
the close relationship between biochemical composition and reproductive cycle of
*Mytilus* species. Widdows and Bayne (1971) observed high contents of glycogen in
*Mytilus edulis* during summer.

In general changes in biochemical components are closely linked to the state of
sexual maturity of the mollusks and to energy supply, either directly from ingested food
or from previously stored reserves (Sastry 1979; Navarro et al., 1989). Carbohydrate,
particularly glycogen are considered to be the main energy source in adult marine
bivalves and are important for gamete formation and maintenance of adult condition
during periods of nutritive stress or in winter (De Zwaan and Zandee, 1984; Gabbott,
1975). Variations in Carbohydrate content show an inverse relationship with the state of
gonad maturity (Beninger, 1982). According to Beninger and Lucas (1984) lipids from
part of the reserves during periods of nutritional deficiency and are an important of
bivalve Oocytes (Holland, 1978). Salaskar and Nayak (2011) studied the nutritional
quality of bivalves *Crassostrea madrasensis* and *Perna viridis* in the kali estuary. Li, Q
Sada and Mori (2000) studied the seasonal biochemical variations in pacific oyster
gonadal tissue during sexual maturation. Marin et al. (2003) reported the variation in
gross biochemical composition, energy value and condition index of venerid clams.
Albentosa *et al.* (2007) studied the response of two species of clams to starvation
physiological and biochemical parameters. Dridi *et al.* (2007) studied the seasonal
variation in weight and biochemical composition of the Pacific oyster. Kang *et al.*
(2007) studied the condition, reproductive activity and gross biochemical composition
of the manila clams. Albentosa *et al.* (2003) studied the absorption of biochemical
components and feeding behavior with natural and carbohydrate rich diets in *Ruditapes
decussatus*. Since no such information is available for the venerid clam of *Gafrarium
pectinatum* and *Gafrarium divaricatum*, the present study was undertaken to elucidate
the seasonal variation of protein, carbohydrate and lipid content in whole body parts and various tissues of both sexes of Gafrarium pectinatum and Gafrarium divaricatum collected from Thondi coast.

8.2. MATERIALS AND METHODS

Clams were collected every month from the study area and brought to the laboratory. They were kept in fiber glass trough with filtered seawater prior to analysis so as to allow them to empty their gut contents. The animals of the size 3.5± 0.5 cm in length and width were selected for biochemical studies. The animals were washed with tap waters. After cutting the mantle, the sex of the animals was identified by the presence of gonad and the muscle tissue was removed from the shell. The whole body tissues was dried in an oven at a constant temperature of 60°C for 24 hours. The dried material was powered and sieved using a bolting silk cloth. The powered meat was used for further analysis. Further, the animals were opened and their meat was weighed separately. They were then disected to remove the different body parts viz Gonad, Mantle, Adductor muscle, Digestive gland and Foot. Different body parts were weighed and kept in an oven at 60°C for 24 hours. The dried components were bought to constant weight, after which the biochemical components were estimated. Five estimations were made in each case and the average was taken in to consideration.

8.2.1. Estimation of Protein

Protein was estimated by Biuret method as modified by Raymond et al. (1964). 20mg of dried material was taken and homogenized in a hand homogenizer with 1 ml of glass distilled water. 2 ml of biuret reagent was added two times and the tissues grinder was cleaned before transferred to the centrifuge tube. After 30 minutes, this sample was centrifuged for ten minutes and the supernatant fluid was transferred in to another tube. Then the calorimetric reading of the supernatant fluid was measured using UV-VIS Double beam Spectrophotometer (UVD-2960) at the wave length of 540um against the blank reading and then the percentage of protein was calculated.
8.2.2. Estimation of Carbohydrates

For the estimation of the total carbohydrate content, the procedure of Dubios et al. (1956) was followed. 20 mg of dried tissue powder was taken and to this 1.0ml of the glass distilled water followed by 1.0 ml of 4% phenol solution and 5 ml of concentrated sulphuric acid were added. After 30 minutes, calorimetric reading was taken in UV-VIS Double beam Spectrophotometer (UVD-2960) at the wave length of 490um against the blank reading and then the percentage of carbohydrate was calculated.

8.2.3. Estimation of Lipids

The chloroform methanol extraction procedure of Folch et al. (1957) was adapted for extracting lipid from the tissue. About 400 mg of powdered tissue was taken in a 10 ml beaker and to this 5ml of Chloroform-Methanol mixture (3:1) was added. The mouth of the beaker was covered by aluminium foil and kept as such overnight for lipid extraction. This extract was filtered using micro filter. The filtrate was taken in a pre weighed beaker and evaporated in hot air oven. The beaker was reweighed with lipid. The difference in weight was taken as total fat content and the percentage was calculated accordingly.

8.3. RESULTS

8.3.1. Protein

In Gafrarium pectinatum, Male, the protein content in the whole animal was fluctuated between 50.4% to 72.50%. The maximum protein value was recorded in May (72.5%) and the minimum value was recorded in Nov (50.4%). Higher percentage of protein values were recorded in gonad (82.3%), mantle (76.5%), adductor muscle (66.4%), digestive gland (68.5%) and foot (60.6%) and the lower percentage of protein values were recorded in whole animal (50.4 %); gonad (58.5%) and mantle (54.5%), adductor muscle (50.5%), digestive gland (54.7%) and foot (40.5%). In all the body parts, low values were found during Monsoon and the high values were found during summer seasons (Fig.35).
In *Gafrarium pectinatum*, Female, the protein content in the whole animal were fluctuated between 55.3% to 79.50%. The maximum protein value was recorded in May (72.5%) and the minimum value was recorded in Dec (55.3%). Higher percentage of protein were recorded in gonad (90.6%), mantle (85.3%), adductor muscle (76.3%), digestive gland (69.5%) and foot(62.6%) and the lower percentage of protein values were recorded in whole animal (55.3 %), gonad (61.7%) and mantle (55.7%), adductor muscle (56.7%), digestive gland (50.3%) and foot (45.5%). In all the body parts, low values were found during Monsoon and the high values were found during summer seasons (Fig.36).

In *Gafrarium divaricatum*, Male, the higher percentage of protein content were recorded in whole animal and various body parts were whole animal (64.5%) gonad (79.3%), mantle (76.5%), adductor muscle (66.1%), digestive gland (61.8%) and foot (59.1%) and the lower percentage of protein values were recorded in whole animal (44.2%); gonad(59.1%) and mantle (56.8%), adductor muscle (50.1%), digestive gland (52.6%) and foot (45.5%). In all the body parts, low values were found during Monsoon and the high values were found during summer seasons (Fig.37).

In *Gafrarium divaricatum*, Female, the higher percentage of protein content were recorded in whole animal and various body parts were in whole animal(69.8%), gonad (88.6%), mantle (80.5%), adductor muscle (78.2%), digestive gland (67.3%) and foot (62.4%) and the lower percentage of protein were recorded in whole animal (50.3 %), gonad (60.3%) and mantle (54.1%), adductor muscle (54.7%), digestive gland (50.3%) and foot (50.6%). In all the body parts, low values were found during Monsoon and the high values were found during summer seasons (Fig.38).

**8.3.2. Carbohydrate**

In *Gafrarium pectinatum*, Male, the carbohydrate content in the whole animal were fluctuated between 4.42% to 7.05%. The maximum carbohydrate value was recorded in May (7.05%) and the minimum value was recorded in Nov (4.42%).
Higher percentage of carbohydrate were recorded in gonad (8.62%), mantle (5.25%), adductor muscle (3.94%), digestive gland (6.83%) and foot (3.51%) and the lower percentage of carbohydrate were recorded in whole animal (4.42%); gonad (5.01%) and mantle (3.41%), adductor muscle (2.00%), digestive gland (3.40%) and foot (2.00%). In all the body parts, low values were found during Monsoon and the high values were found during summer seasons (Fig.39).

In *Gafrarium pectinatum*, Female, the carbohydrate content in the whole animal were fluctuated between 4.28% to 7.60%. The maximum carbohydrate value was recorded in May (7.60%) and the minimum value was recorded in Nov (4.28%). Higher percentage of carbohydrate were recorded in gonad (9.22%), mantle (4.56%), adductor muscle (3.44%), digestive gland (5.53%) and foot (3.56%) and the lower percentage of carbohydrate were recorded in whole animal (4.28%), gonad (5.51%), mantle (3.21%), adductor muscle (2.21%), digestive gland (3.14%) and foot (2.23%). In all the body parts, low values were found during Monsoon and the high values were found during summer seasons (Fig.40).

In *Gafrarium divaricatum*, Male, the higher percentage of carbohydrate content were recorded in whole animal and various body parts were: whole animal (6.45%) gonad (7.62%), mantle (5.85%), adductor muscle (4.44%), digestive gland (6.31%) and foot (3.51%) and the lower percentage of carbohydrate were recorded in whole animal (4.02%); gonad (4.52%), mantle (4.80%), adductor muscle (2.11%), digestive gland (2.01%) and foot (2.10%). In all the body parts, low values were found during Monsoon and the high values were found during summer. (Fig.41)

In *Gafrarium divaricatum*, Female, the higher percentage of carbohydrate content were recorded in whole animal and various body parts were in whole animal (6.60%), gonad (8.22%), mantle (3.56%), adductor muscle (3.54%), digestive gland (5.53%) and foot (3.89%) and the lower percentage of carbohydrate were recorded in whole animal (3.28%); gonad (4.42%) and mantle (2.00%), adductor muscle (2.11%),
digestive gland (2.14%) and foot (1.23%). In all the body parts, low values were found during Monsoon and the high values were found during summer. (Fig.42)

8.3.3. Lipid

In *Gafrarium pectinatum*, Male, the lipid content in the whole animal was fluctuated between 3.13% to 6.45%. The maximum lipid value was recorded in June (6.45%) and the minimum value was recorded in Nov (3.13%). Higher percentage of lipid were recorded in gonad (5.39%), mantle (5.33%), adductor muscle (3.76%), digestive gland (6.73%) and foot (3.49%) and the lower percentage of lipid were recorded in whole animal (3.31%); gonad (3.01%) and mantle (3.23%), adductor muscle (2.04%), digestive gland (3.44%) and foot (2.09%). In all the body parts, low values were found during Monsoon and the high values were found during summer season (Fig.43).

In *Gafrarium pectinatum*, Female, the lipid content in the whole animal was fluctuated between 3.22% to 5.72%. The maximum lipid value was recorded in April (5.72%) and the minimum value was recorded in Dec (3.22%). Higher percentage of lipid were recorded in gonad (5.28%), mantle (4.28%), adductor muscle (3.34%), digestive gland (5.75%) and foot (3.85%) and the lower percentage of lipid were recorded in whole animal (3.22%); gonad (3.01%), mantle (2.51%), adductor muscle (2.01%), digestive gland (3.21%) and foot (1.29%). In all the body parts, low values were found during Monsoon and the high values were found during summer season (Fig.44).

In *Gafrarium divaricatum*, Male, the higher percentage of lipid content were recorded in whole animal and various body parts were : whole animal (5.45%) gonad (5.39%), mantle (3.56%), adductor muscle (3.63%), digestive gland (5.73%) and foot (3.90%) and the lower percentage of lipid were recorded in whole animal (2.13 %); gonad (2.42%), mantle (2.23%) adductor muscle (2.28%), digestive gland (3.24%) and
foot (2.09%). In all the body parts, low values were found during Monsoon and the high values were found during summer season (Fig. 45).

**In Gafrarium divaricatum.** Female, the higher percentage of lipid content were recorded in whole animal and various body parts were in whole animal (3.72%), gonad (5.58%), mantle (3.54%), adductor muscle (3.27%), digestive gland (4.63%) and foot (2.95%) and the lower percentage of lipid were recorded in whole animal (2.29%), gonad (3.42%), mantle (2.61%), adductor muscle (2.31%), digestive gland (3.23%) and foot (1.27%). In all the body parts, low values were found during Monsoon and the high values were found during summer season (Fig. 46).

### 8.4. DISCUSSION

Giese *et al.* (1958) found that in Vertebrate transfer of reserve of nutrient from the storage sites takes place to the gonadal synthetic centers during gametogenesis. Giese (1969) stated that the biochemical levels varied considerably at different times during the year and the variations in protein level from month to month did not show any relation to the reproductive season in *Tivela stultorum* and may depend upon the difference in nutrients conditions. Ansell (1974, a,b & c) recorded a decrease in lipid and protein nitrogen levels during spawning period in *Abra alba*, *Chlamys septemradiata* and *Nucula sulcata*. Definite changes in the biochemical constituents in clams were demonstrated by Balasubramanian and Natarajan (1988a) in *Meretrix casta*, George John (1980) in *Anadara rhombea* and Jayabal (1984) in *Katelysia opima*. A very good relationship between the maturation and spawning of the clams with biochemical constituents was evident.

In the present study, the Gonads of male and female of both species Gafrarium showed higher protein values than other organs. Thus the gonad seems to serve as a storage organ of protein in *Gafrarium pectinatum* and *Gafrarium divaricatum*. High protein value observed in summer could be due to intense proliferation of gonad and in monsoon the low protein value may be due to spawning activity. Another peak value in
August coincided with secondary peak of breeding activity and the low values in subsequent month due to spawning. Nagabushanam and Deshmukh (1974) reported that the variation in the protein level of body components of Meretrix meretrix did not show any relation to the reproductive cycle but the fat content decreased in gonad during spawning.

In the present study, carbohydrate values were high in the Gonads of male and female of both species of Gafrarium. Thus the gonad seems to serve as a storage organ of carbohydrate in Gafrarium pectinatum and Gafrarium divaricatum. High carbohydrate value observed in summer could be due to intense proliferation of gonad, but were very low, when the gonad was ripe and in monsoon the low protein value may be due to spawning activity. Giese (1969) observed that in Tivela stultorum, the values of carbohydrate were high in gonad during proliferation stages. He reported that carbohydrate may act as a storage material in Tivela stultorum.

In the present study, Lipid content was high in gonad, mantle and digestive gland of male and female of both species of Gafrarium pectinatum and Gafrarium divaricatum. High lipid values were observed in summer may be due to maturity and the low value may be due to spawning activity. George John (1980) suggested that digestive gland may be considered as a storage organ of lipid in Anadara rhombea from vellar estuary. Similar observations were also made on Chlamys septemradiata (Ansell, 1974b), Tivela stultorum (Giese, 1969) and Chlamys opercularis (Taylor and Venn,1979). The increase in fat content during the period of gonad development was reported by Venkataraman and Chari (1951) in Meretrix meretrix and Joshi and Bal (1965) in Katelysia marmorata.
Fig. 35. Monthly variation of Protein - *Gafrrarium pectinatum* – Male

Fig. 36. Monthly variation of Protein - *Gafrrarium pectinatum* – Female

Fig. 37. Monthly variation of Protein - *Gafrrarium divaricatum* – Male
Fig. 38. Monthly variation of Protein - *Gafrarium pectinatum* – Female

Fig. 39. Monthly variation of Carbohydrate content - *Gafrarium pectinatum* – Male

Fig. 40. Monthly variation of Carbohydrate content - *Gafrarium pectinatum* – Female
Fig. 41. Monthly variation of Carbohydrate content - *Gafrarium divaricatum* – Male

Fig. 42. Monthly variation of Carbohydrate content - *Gafrarium divaricatum* – Female

Fig. 43. Monthly variation of Lipid content - *Gafrarium pectinatum* – Male
Fig. 44. Monthly variation of Lipid content - *Gafrarium pectinatum* – Female

Fig. 45. Monthly variation of Lipid content - *Gafrarium divaricatum* – Male

Fig. 46. Monthly variation of Lipid content - *Gafrarium divaricatum* – Female