CHAPTER VII
BIOCHEMICAL COMPOSITION

7.1 INTRODUCTION

In India coastal and estuarine areas sustain vast resources of molluscs. Bivalve molluscs, a potential source of valuable proteins, carbohydrates and minerals, are abundantly available in the country. Among them edible mussels are of great potential because of their high productivity. These have been exploited by the people of the coastal areas from time immemorial for food and also for their shells. An understanding of its nutritional aspects can lead to better utilisation of the resources. Seasonal cycles of the variation in the biochemical constituents like protein, carbohydrate and lipid are generally attributed to the complex interaction between environmental parameters, food availability, growth and reproductive activity (Bayne, 1976; Sastry, 1979; Gabbot, 1976, 1983). In general, the energy is stored prior to gametogenesis when food is abundant and is utilised in the production of gametes when metabolic demand is high (Bayne, 1976; Gabbot, 1975). The study of variation in energy storage in the form of protein, glycogen and lipid would help in understanding the ecology and overall economy of the species. A sound knowledge of variations in biochemical composition in different stages of growth is essential so that it enables the exploitation of bivalves when their nutritional value is greatest.

7.2 REVIEW

Studies on the biochemical composition of different species of bivalves have, because of their importance as food and their role in the economy, received the attention of scientific workers in several parts of the world.

Giese et al. (1967) suggested that the analysis of various body components for biochemical constituents would be more informative to elucidate the mobilisation of the tissue reserves to the gonad during gametogenesis. Nagabhushanam and Deshmukh (1974) estimated protein, fat, glycogen and water content of different body components such as adductor muscles, digestive glands, foot, gills, gonads, mantle, siphon and rest of the body and whole tissue of *Meretrix meretrix*. Ansell (1974b) analysed carbohydrate, nitrogen, lipid, carbon and water content of gonad, adductor muscle and mantle tissue of *Chlamys septemradiata* from the Clyde Sea area. Thompson (1977) has
separated gonad and somatic tissue of *Placopecten magellanicus* and estimated carbohydrate, lipid, protein and ash content. Salih (1977) observed variation in biochemical composition of different tissue components like gonad, adductor muscle and body entire of *Meretrix casta*. Pieters *et al.* (1979) investigated the changes in glycogen, protein and total lipid level of mantle and total tissue of *Mytilus edulis* and Zurberg *et al.* (1978) analysed gills, mantle, posterior adductor muscle, hemolymph and combined remaining tissues of this mussel for glycogen, lipid, protein and free amino acids. Taylor and Venn (1979) have given an account of the seasonal variation in weight and biochemical composition of the tissues like adductor muscle, gonad and other tissues of *Chlamys opercularis* from the Clyde Sea area. Seasonal changes of protein and nitrogen levels in the adductor muscle, gill and midgut gland of the clam *Tapes philippinarum* were reported by Adachi (1979). Stephen (1980b) analysed variation in water, protein, carbohydrate, lipid and ash content in the adductor muscle, mantle and gonad of *Crassostrea madrasensis*. Barber and Blake (1981) gave an account of the biochemical changes in the adductor muscle, mantle, digestive gland and gonad tissues of *Argopecten irradians concentricus*. Thangavelu and Sanjeevaraj (1988) estimated protein, lipid, carbohydrate and water content of the different body components like mantle, gill, adductor muscle, gonad and hepatopancreas of the oyster, *Crassostrea madrasensis*. Katticaran (1988) reported seasonal biochemical changes in foot, mantle, adductor muscle, gill, digestive gland and gonad of *Sunetta scripta*.

Among the above studies only in a few cases wet tissue were used for the estimation of different biochemical constituents. A few works carried
out with wet tissue are on Martesia fragilis (Srinivasan and Krishnaswamy, 1963), Crassostrea virginica (Galtsoff, 1964), Lamellicdens marginalis (Ahmed et al., 1978), Tapes decussatus and T. philippinarum (Beninger and Lucas, 1984), Villorita cyprinoides var. cochinensis (Sathyanganathan et al., 1988) etc.

The mussel M. senhusia occur in good quantity in some areas of Cochin waters and it is utilised as poultry feed and fertilizer. From the studies on maturity stages of this mussel it is revealed that most of the stages of the development could be obtained throughout the year. Three maturity stages recognised are developing, mature and spawning. Animals with gonads in these stages were analysed for different biochemical components like protein, lipid and glycogen. Thus the present study has been undertaken to elucidate the biochemical make up of different body components like mantle-gonad, foot and whole tissue of the mussel M. senhusia with regard to sex and gonad condition.

7.3 MATERIALS AND METHODS

Collection were made at an interval of fifteen days for a period from April 1989 to April 1990. Mussels within the length range of 10-24 mm selected for the study were cleaned, and acclimated in filtered water of habitat salinity for two days. Individuals of two sexes were separated by the examination of gonad smears microscopically.

Wet weight was measured after washing the tissues with distilled water and removing the excess water with filter paper. In order to determine the dry weight, the weighed samples were dried at 60-80°C until a stable
weight was reached. From these the water content was calculated by the following formula:

\[
\frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100 = \% \text{ wet weight}
\]

For biochemical estimations several individuals of the same sex with similar gonad condition were pooled, as the weight of the component organs dissected from individual specimen was too small to meet the requirement of different estimations. Wet tissues were used for all the estimations. Total tissue and different tissue components such as mantle+gonad and foot were then used for the analysis of glycogen, protein and lipid.

For the estimation of glycogen the method followed was that of Montgomery (1957). A quantity of 0.2 ml of the tissue extract was added to 1.0 ml of 10% trichloroacetic acid. This mixture was centrifuged for 10 minutes at 2500 rpm. To 1 ml of the supernatant added 2 ml of 95% ethyl alcohol. This mixture was kept undisturbed for about 12 hours in a refrigerator, and then centrifuged at 2500 rpm for 15 minutes. The supernatant was decanted and 0.1 ml of 80% phenol and 2 ml of distilled water were added to the pellet. To this 5 ml sulphuric acid was added and mixed well. After 30 minutes optical density of the colour developed was measured at 490 nm. Calibration curve was prepared using glucose as standard.

Lowry's method (Lowry et al., 1951) was used for the determination of total protein. To 1 ml of 10% trichloroacetic acid added 0.2 ml of the tissue extract and centrifuged at 2500 rpm for 15 minutes. The precipitate was dissolved in 1 ml of 0.1 N sodium hydroxide; 5 ml of alkaline copper
reagent was added to it and mixed well. After 10 minutes, 0.5 ml of Folin's phenol reagent was added and shaken well. After 30 minutes the intensity of the blue colour developed was measured spectrophotometrically at 500 nm. Here bovine serum albumin was used to prepare the standard.

Lipid levels in the tissue was determined by the method of Barnes and Blackstock (1973). Tissue samples were extracted with chloroform methanol mixture. To 1 ml of this extract, 1 ml of methanol, 2 ml of chloroform, 2 ml of chloroform-methanol mixture and 0.2 volume of 0.9% sodium chloride were added one by one, mixed well and allowed to stand for a few hours. The lower phase was separated and transferred into a clean test tube and made up the volume to the original quantity of chloroform added before; 0.5 ml of the extract was taken and dried in a vacuum desiccator over silica gel; 0.5 ml of concentrated sulphuric acid was added to this, mixed well. The test tube was plugged with non-absorbent cotton and placed in boiling water bath for 10 minutes. After cooling, 0.2 ml of the acid digest was taken, added 5 ml of phosphovanillin reagent, mixed well and allowed to stand for half an hour. After this, optical density of the red colour developed was measured at 520 nm. Cholesterol was used for the preparation of the standard curve.

In all cases the values are expressed as \( \mu g/\text{mg} \) wet weight. The experiments repeated with several samples.

7.4 RESULTS

The biochemical composition of the total tissue, mantle and foot were analysed on the basis of gametogenesis. The data thus obtained for the three
stages, that is, developing, mature and spawning are presented in Tables 25-27 and in Figures 32-35.

Total tissue

In the total tissue, the water content (expressed in percentage wet weight) in the spawning stage was highest (89.25% WW) (Table 25) when compared to female tissue. In maturing condition it was little less (86.03%) than that of the developing stage (87.40%). The water level again increased in the spawning stage. In general there is no appreciable variation in water content in different stages. The average protein level (expressed in µg/mg wet weight) obtained showed high value (56.61 µg/mg) in developing stages. From this level it decreased to 40.18 µg/mg in mature condition, and 34.22 µg/mg in spawning stage. The lowest protein level was observed in spawning stage. Glycogen and lipid also showed the same trend. These two components showed a high value in developing stage (glycogen: 25.71 µg/mg and lipid: 26.60 µg/mg) and low value (glycogen: 12.93 µg/mg and lipid: 18.22 µg/mg) in spawning stage. In both the stages the lipid component was little more than the glycogen content.

Like the male tissue, in female also the water level decreased as the development proceeded. Here the highest level of water content (88.90%) could be noticed in developing stage (Table 25) and the lowest level was found in mature condition (86.23%). But in the spawning stage it was again found to be increasing. Other components like protein, glycogen and lipid showed a decreasing trend as the development proceeded. Glycogen level showed a slight increase (27.14 µg/mg) in females.
Table 25. Details: water content (% wet weight), protein, glycogen and lipid (µg/mg wet weight) components of total tissue in *M. senhausia* during three phases of gametogenesis
DG = Developing, M = Mature, SG = Spawning

<table>
<thead>
<tr>
<th>Sex</th>
<th>Gonadal condition</th>
<th>Water content</th>
<th>Protein M±SD</th>
<th>Glycogen M±SD</th>
<th>Lipid M±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>DG</td>
<td>87.40</td>
<td>56.61±5.09</td>
<td>25.71±8.22</td>
<td>26.60±5.71</td>
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<tr>
<td>Female</td>
<td>DG</td>
<td>88.90</td>
<td>56.85±3.80</td>
<td>27.14±9.47</td>
<td>23.22±5.65</td>
</tr>
<tr>
<td>Male</td>
<td>M</td>
<td>86.03</td>
<td>40.18±10.57</td>
<td>15.08±5.90</td>
<td>18.99±4.27</td>
</tr>
<tr>
<td>Female</td>
<td>M</td>
<td>86.23</td>
<td>37.46±12.78</td>
<td>18.65±11.89</td>
<td>18.95±5.61</td>
</tr>
<tr>
<td>Male</td>
<td>SG</td>
<td>89.25</td>
<td>34.22±10.75</td>
<td>12.93±4.62</td>
<td>18.22±4.78</td>
</tr>
<tr>
<td>Female</td>
<td>SG</td>
<td>87.38</td>
<td>34.18±17.09</td>
<td>15.94±5.83</td>
<td>14.32±4.32</td>
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</tbody>
</table>
Figure 35. Variation in protein, glycogen and lipid components in the total tissue (M - male and F - female) of M. senhausia during different developmental stages (dg - developing, M - mature and Sg - spawning).
Table 26. Details: water content (% wet weight), protein, glycogen and lipid (µg/mg wet weight) components of mantle+gonad tissue in M. senhausia during three phases of gametogenesis. DG = Developing, M = Mature, SG = Spawning

<table>
<thead>
<tr>
<th>Sex</th>
<th>Gonadal condition</th>
<th>Water content</th>
<th>Protein</th>
<th>Glycogen</th>
<th>Lipid</th>
</tr>
</thead>
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<tr>
<td>Male</td>
<td>DG</td>
<td>94.10</td>
<td>56.21±9.83</td>
<td>22.95±10.18</td>
<td>24.67±9.13</td>
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<tr>
<td>Female</td>
<td>DG</td>
<td>89.70</td>
<td>58.42±12.45</td>
<td>23.39±13.17</td>
<td>26.13±6.11</td>
</tr>
<tr>
<td>Male</td>
<td>M</td>
<td>82.94</td>
<td>47.52±13.94</td>
<td>17.63±6.66</td>
<td>23.03±6.77</td>
</tr>
<tr>
<td>Female</td>
<td>M</td>
<td>83.13</td>
<td>45.67±17.88</td>
<td>15.29±9.74</td>
<td>25.71±5.69</td>
</tr>
<tr>
<td>Male</td>
<td>SG</td>
<td>81.30</td>
<td>41.42±12.02</td>
<td>17.31±6.90</td>
<td>17.99±4.18</td>
</tr>
<tr>
<td>Female</td>
<td>SG</td>
<td>79.85</td>
<td>41.84±13.95</td>
<td>13.56±4.09</td>
<td>19.33±4.47</td>
</tr>
</tbody>
</table>
Figure 36. Variation in protein, glycogen and lipid components in mantle + gonad of M. senhausia (M - male and F - female) during different developmental stages (Dg - developing, M - mature and Sg - spawning).
Mantle+Gonad

In the developing stage in male, mantle+gonad maintained a slightly higher water level (94.1%) than in the other body components (Table 26). As gonad maturation proceeded water level showed a gradual decrease. Comparatively higher water decline was observed in mature condition (82.94%). Protein, glycogen and lipid showed a decrease towards the spawning stage. Much higher decrease in protein and glycogen content could be observed in mature condition (Protein from 56.21 to 47.52 μg/mg and glycogen from 22.95 to 17.63 μg/mg). Only slight decrease could be noticed in the case of lipid in mature condition (from 24.67 to 23.03 μg/mg). But in later stage the decrease of lipid was much higher (17.99 μg/mg) when compared to the previous stage. In the case of protein and glycogen also the lowest level could be noticed in spawning condition (protein: 41.42 and glycogen: 17.31 μg/mg).

Protein, glycogen and lipid showed slightly higher value in the tissue of females in developing stage (58.42, 23.39 and 26.13 μg/mg), but the water content was lower (89.7%). All these components showed a decreasing trend as gonad maturation proceeded. Water, protein and glycogen contents decreased to 83.13%, 45.67 μg/mg and 15.29 μg/mg respectively in mature stage, the decrease in lipid level was only slight (25.71 μg/mg). In the spawning stage all these components showed further decrease, which in the case of lipid was little more (19.33 μg/mg) than in others. Water, protein and glycogen levels obtained in this stage were 79.85% WW, 41.84 μg/mg and 13.56 μg/mg respectively.
Table 27. Details: water content (% wet weight), protein, glycogen and lipid (µg/mg wet weight) components in foot tissue of *M. senhausia* during three phases of gametogenesis

DG = Developing, M = Mature, SG = Spawning

<table>
<thead>
<tr>
<th>Gonadal condition</th>
<th>Water content</th>
<th>Protein</th>
<th>Glycogen</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>DG</td>
<td>80.80</td>
<td>88.08±24.00</td>
<td>27.74±14.78</td>
<td>27.99±8.35</td>
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<td>M</td>
<td>73.08</td>
<td>77.99±25.12</td>
<td>24.14±10.45</td>
<td>25.54±4.29</td>
</tr>
<tr>
<td>SG</td>
<td>74.00</td>
<td>70.84±20.13</td>
<td>14.43±3.74</td>
<td>19.75±4.77</td>
</tr>
</tbody>
</table>
Figure 37. Variation in protein, glycogen and lipid components in foot tissue of *M. senhausia* during different developmental stages (Dg - developing, M - mature and Sg - spawning).
Figure 38. Variation in water content in different tissues like total tissue (male (MT) and female (FT)), Mantle + gonad (male (MG) and female (FG)) and foot (Ft) during different development stages (Dg - developing, M - mature and Sg - spawning).
Foot

Owing to the paucity of enough quantity of foot tissue from each sex, the foot tissue from several mussels were pooled irrespective of sex for this study. In the foot tissue the water content was low when compared to the other tissues (Table 27). It showed variation according to the development of the gonad. High value of water content (80.80%) was observed in the developing stage and low value (73.08%) in mature stage, followed by a slight increase (74%) in the spawning stage. Among the different tissue components, comparatively high protein value (88.08 µg/mg) could be seen in the foot tissue. Protein level showed a decrease with the development of the gonad. Glycogen and lipid also showed similar trend. In the case of glycogen and lipid only slight decrease could be observed in the first two stages (glycogen from 27.74 to 24.14 µg/mg and lipid from 27.99 to 25.54 µg/mg), but in later stages the decrease (14.43 µg/mg and 19.75 µg/mg respectively) in these components were little more.

7.5 DISCUSSION

Variations in the biochemical composition are influenced by different factors like hydrographic conditions, availability of food, growth and reproduction. Knowledge of the reproductive cycle is essential for interpretation of variations in biochemical composition of the tissues (Taylor and Venn, 1979). Observations on M. senhausia revealed that the main period of gonad proliferation and maturation occurred between December and March. Intense spawning activity was observed during March-April with intermittent spawning throughout the year. So the population apparently consists of individuals
with ripe gametes throughout the year. Energy reserves like protein, lipid and glycogen showed marked quantitative changes during different developmental stages. The variation in water content and biochemical composition are associated with the reproductive activity in relation to storage and utilisation of food reserves during growth and reproduction.

The water content in the gonad tissue was higher when compared to the other tissues, and it showed gradual decrease with development. Ansell (1974a) stated that the water content of the bivalve tissue may give an indication of the time of spawning. In the case of total tissue and foot the water level which is comparatively low in the latter showed a decrease in mature stage, and then an increase in spawning condition. High water content was observed during spawning in Villorita cyprinoides (Ansari et al., 1981) and Villorita cyprinoides var. cochinensis (Nair and Shynamma, 1975a). Ansell (1974b) observed lowest water level in the ripe gonad of Chlamys septemradiata, and a considerable increase during spawning period. Salih (1977) observed a low water level in the developing stage in Meretrix casta.

In the present study it is revealed that protein was the major fraction that shows maximum difference in various stages of development. Here it presents a decreasing trend as maturation proceeds. Pieters et al. (1978) observed a decrease of protein in the ripening stage of Mytilus edulis. But Giese et al. (1967) pointed out that in Tivela stultorum tissue protein level remained constant throughout the year. Nagabushanam and Deshmukh (1974) observed an increase in protein content of Meretrix meretrix in mature condition. Ansari et al. (1981) suggested that the level of protein during
gametogenesis in *Villorita cyprinoides* is decreased during breeding season and it again rises to a second peak before the second spawning. In *Sunetta scripta* Katticaran (1988) observed a peak protein value at the commencement of spawning and a gradual depletion during spawning. In *M. senhau sia* depletion of protein value in foot tissue during maturation can be explained as a consequence of mobilisation of protein reserves for utilisation during gametogenesis. Mobilisation of protein from different tissues has been reported in *Tapes philippinarum* (Adachi, 1979) and *Mytilus edulis* (Gabbot and Bayne, 1973).

The carbohydrates in bivalves comprised mainly of glycogen (Gabbot and Bayne, 1973) and large amounts are stored in adductor muscle (Taylor and Venn, 1979), mantle (de Zwaan and Zandee, 1972), gonad (Sastry, 1979; Gabbot, 1983) and digestive gland (Thompson, 1977; Barber and Blake, 1981). In the present study the glycogen content was slightly higher in the females. It may be attributed to higher biochemical budget required for oogenesis. The glycogen level showed decreasing trend with the advancement of gametogenesis. This may be due to a massive conversion of glycogen for the development of gametes. This is in agreement with Galtsoff (1964) who observed a high carbohydrate level in immature oyster, *Crassostrea virginica* and it declined steeply as gametes were formed in the gravid animals. Giese et al. (1967) showed that gonads of *Tivela stultorum* had the least carbohydrate storage when mature gametes were present. Nagabhushanam and Deshmukh (1974) also noticed a high level of glycogen during the period of gonad development and a fall when the gonads were in mature condition. Similar results were again observed in *Mytilus edulis* (Pieters et al., 1978) and *Crassostrea madrasensis* (Stephen, 1980b). Katticaran (1988) observed that total carbohydrate
level in the maturing gonad in *Sunetta scripta* showed a decreasing trend with the advancement of gametogenesis with the lowest level at the early spawning. But Salih (1977) observed an increase in glycogen during spawning period in *Meretrix casta*. He suggested that the rise may be only an indication of normal metabolic activity consequent on the draining of excess water from the tissues and on the rise in salinity of the surrounding water. In *Donax incarnatus* Nagabhushanam and Talikhedkar (1977b) noticed highest glycogen content during mid season of gonad ripening. The difference in the storage and utilisation of glycogen reserves reflects the complex interaction between growth and reproductive cycle (Taylor and Venn, 1979).

In the present study lipid component was slightly greater than glycogen content. But its utilization was less during the period of maturation, especially in the mantle-gonad tissue, but later at the commencement of spawning the decline in the lipid content was more when compared to the earlier stage. Salih (1977) reported a fall in lipid content with spawning in *Meretrix casta*. Pieters et al. (1978) noticed a rapid decrease of lipid during ripening in *Mytilus edulis*. In *Meretrix meretrix* Nagabhushanam and Deshmukh (1974) observed low values of lipid due to discharge of gametes during spawning, but a higher value was reported when the gonads were fully ripe. In fully mature stage a peak lipid value was observed in *Donax cuneatus* (Nagabhushanam and Talikhedkar, 1977b) and *Villorita cyprinoides* var. *cochinensis* (Lakshmanan and Nambisan, 1980). But Ansari et al. (1981) found no variation in the lipid content in *Villorita cyprinoides*.

Thus the amount of protein, lipid and glycogen showed variation in the present study. These three components showed a decrease with the
advancement of gametogenesis in all the tissue components studied. In the case of gonad, utilisation of glycogen was more in mature stage than that of lipid. This may be due to the fact that besides using as an energy source, glycogen reserves are converted into lipid during gametogenesis which are stored in the ripening gametes as reserves to be used subsequently in early embryonic development. During later stages, that is in spawning condition, the depletion of lipid is more with the release of mature gametes. According to Giese et al. (1967) the gonads of the clam accumulates their nutritional reserves for gametogenesis independently of other body components. In the present study the utilization of the energy reserves for the gametogenesis is evident from the observed decrease in protein, lipid and glycogen contents. The water content also showed a decreasing trend in gonad. But an inverse relationship between organic constituents and water level in total tissue and foot is observed during spawning period. This increase may be due to the release of gametes and depletion of other stored organic substances from different tissues of the body. Thus it can be concluded that the water, protein, glycogen and lipid levels vary according to different developmental stages.