CHAPTER VII
PROXIMATE BIOCHEMICAL COMPOSITION
Aquatic ecosystems are regarded as a potential food source for human and animals. The development in agriculture technology has increased the production of food grains, nevertheless, this has been accompanied by phenomenal increase in human population. Thus, the major question is whether the increase in food production can keep up with the exponential growth of the human population. Besides hunger, the deficiency of essential amino acids (protein), fatty acids (lipid), vitamins etc. seems to be a matter of concern especially in developing countries. Deficiency of protein and vitamins, leads to mental and physical disorder and predisposes to various kinds of diseases. The biological value of fish and marine animals is highly rated by nutrition physiologists. Fish protein contains relatively high percentage of methionine, lysine and tryptophan (Meseck, 1962). Moreover, fish lipid, vitamins and minerals are considered as valuable source for nutrition.

Generally, the whole body biochemical composition is an indication of fish quality. Therefore, proximate composition of a species helps to assess its nutritional value in comparison to other organisms. Proximate composition is also required by the nutrition experts and individuals interested in caloric content of the food for weight control. Recently, cardiologists are recommending the use of fish in food to obtain adequate protein without excessive fatty acids and lipid (Dyerberg, 1986; Kinsella, 1991). Industries involved in the production of
balanced diet as well as fast foods, need to know proximate composition of the basic ingredients of the food. Further, as a biologist it is important to understand the dynamics of organic constituents with reference to change in season, size, reproductive stage and sex, as the former seems to depend on the latter, to a greater extent (Phillips et al., 1966; Groves, 1970; Somvanshi, 1983; Sinha and Pal, 1990).

Studies on fish culture have also paid attention to the effects of dietary organic constituents on the number and viability of eggs and brood stock body composition (Smith et al., 1979; Takeuchi et al., 1981; Washburn et al., 1990). The proximate composition of fish ovaries has been well documented (Apparao, 1967; Devauchelle et al., 1988; Washburn et al., op. cit.). In addition, the influence of environmental pollutants has also been well known (Bano et al., 1981; Sahib et al., 1983; Vishwaranjan et al., 1988; Jyoti et al., 1989; Somanath, 1991; Sashtry and Dasgupta, 1991).

The present study was undertaken to elucidate the dynamics of biochemical components of muscle and ovary of E. suratensis with reference to different maturity stages, size/age and seasons.

7.2 MATERIALS AND METHODS

Male and female specimens of E. suratensis were collected
separately every month over a period of 16 months, from February 1991 to May 1992. Body muscle samples (free from skin and scales) from five to six fish were pooled together and used for the analysis of biochemical components. Similarly, a composite sample of fish representing different sizes, maturity stages of fish and ovary was taken to study the dynamics of biochemical constituents in these tissues. The moisture content was estimated by drying the pre-weighed wet sample at 70 °C until a constant weight was obtained. The difference in weight was calculated and expressed as percentage moisture content of the sample. The dried samples were then finely powdered in a mortar and pestle and stored in desiccator until further analysis.

Protein was estimated following the method of Herbert et al. (1971) with slight modifications to suit the fish samples. The care was however taken not to deviate from the basic principle of the analysis. A random sample of constant weight (5.0 mg) was extracted with different volume of NaOH viz, 1, 2, 4, 6, 8 and 10 ml to find out the suitable volume of NaOH for the maximum extraction of protein. The results of this analysis (Fig. 7.1) revealed that a volume of 6 ml to 10 ml were suitable for extraction. Therefore, this volume of NaOH was used for the extraction of protein from fish samples. To a known sample (4.4 to 5.1 mg), 10 ml of 1N NaOH was added and sample was extracted at 80 °C for half an hour in a water bath. Thereafter, it was cooled at room temperature, and neutralised with 10 ml of 1N HCl. The extracted sample was centrifuged at 2000 rpm for 10 minutes, and an aliquot of the sample (10 ml) was further diluted.
Fig. 7.1 Evaluation of the efficiency of sodium hydroxide for the extraction of protein.

Fig. 7.2 Regression analysis between protein concentration and absorbency.
with equal volume of distilled water. From this diluted sample, 1 ml was taken and treated with 2.5 ml of mixed reagent (Carbonate-tartrate-copper) and 0.5 ml of 1N Folin's-Ciocalteau reagent. After 30 minutes, sample absorbency was read at 750 nm in Beckman DU-6 Spectrophotometer. Appropriate blanks and standards (Bovine-serum-albumin) were treated similarly to obtain a standard calibration curve (Fig. 7.2). All the results expressed as mg/g of dry sample.

Carbohydrate was estimated by using the phenol sulphuric acid method (Dubois et al., 1956) as suggested by Hitchcock (1977). However, prior to final analysis a random sample of constant weight (5.0 mg) was treated with different volumes viz, 1, 2, 4, 6 and 8 ml of 80% sulphuric acid in order to find out the optimum requirement of sulphuric acid to extract the absolute carbohydrate from the sample. It is clear from Fig. 7.3, that 2 ml of 80% sulphuric acid is the best suited volume to extract carbohydrate from 4-5 mg of dried fish sample and the same was used throughout the analysis. A known sample of 4.4 to 5.2 mg was treated with 2 ml of 80% sulphuric acid and was allowed to digest for 21 hours at room temperature. 2 ml of phenol reagent (5%) followed by 5 ml of concentrated sulphuric acid were added to the digested sample and allowed to cool. The mixture was centrifuged and absorbency measured at 490 nm. Analar grade D-glucose was used as standard to draw a straight line (Fig 7.4). All the concentrations are expressed as mg/g dry sample.

Lipid was estimated by the method of Parsons et al. (1984). A known sample (5.0 - 7.5 mg) was homogenized in 8 ml of
Carbohydrate concentration (mg/g) vs. Volume of sulphuric acid

- Carbohydrate  ○ Absorbency

*Fig. 7.3* Evaluation of the efficiency of sulphuric acid for the extraction of carbohydrate.

Absorbency vs. Glucose concentration (µg)

*Fig. 7.4* Regression analysis between carbohydrate concentration and absorbency.
chloroform-methanol-distilled water mixture (1:2:0.8 v/v). The extract was filtered through an ignited (450 °C for 3 hours) GF/C filter paper, and the filtrate was transferred to a separating funnel. To this, 2 ml of distilled chloroform and 4 ml of distilled water were added and the entire mixture was shaken for 10 minutes. The chloroform layer was separated and the procedure repeated with leftover sample. The collected chloroform layer was evaporated to dryness in vacuum. The extracted lipid was treated with 3 ml of 0.15% of potassium dichromate and then kept for boiling in water bath for 15 minutes. To this, 4 ml of distilled water was added and upon cooling the absorbency was read at 440 nm. The blanks were treated similarly along with each set of analysis. The standard (stearic acid) was prepared following the same procedure (Fig. 7.5.1). For the analysis of gonad samples the second standard was prepared by taking higher concentration of stearic acid and by increasing the volume of potassium dichromate to 10 ml followed by the dilution with 10 ml of distilled water (Fig. 7.5.2) since the lipid concentration in gonads was higher than the oxidizing capability of 3 ml of dichromate. The concentrations are expressed as mg/g dry sample.

Organic carbon in the sample was determined following the method of El Wakeel and Riley (1957). A known weight (10-12 mg) of the sample was taken in a wide mouth test tube and treated with 10 ml of chromic acid. The samples were then boiled in water bath for two and half hours. After cooling, the content was transferred to a 500 ml conical flask containing 200 ml of distilled water. Four to six drops of ferrous phenanthroline
Fig. 7.5.1 Regression analysis between lipid concentration and absorbency.

Fig. 7.5.2 Regression analysis between lipid concentration and absorbency.
indicator were added to the sample and titrated against 0.2N ferrous ammonium sulphate until a pink colour just persisted. Blanks were also carried out in the similar manner with each set of analysis. One ml of 0.2N ferrous ammonium sulphate is equivalent to $1.05 \times 0.6$ mg of carbon.

The ash content was estimated by ashing the pre-weighed samples (about 500 mg) at 600°C in a muffle furnace for 6 hours. The difference in weight was calculated and the values are expressed as mg/g dry weight.

Calorific content was calculated by multiplying with a factor 5.7 for protein, 4 for carbohydrate and 9.3 for lipid, respectively (Prosser and Brown, 1965).

Concentration of protein, carbohydrate and lipid was calculated using the following formula:

$$\frac{E \times F}{mg/g} = \frac{---------}{W \ (g)}$$

where,

$E$ = Extinction co-efficient

$F$ = Factor derived from standard

$W$ = Weight of sample in g

7.3 RESULTS

7.3.1 Variation in Gonadal Maturity Stages: Four maturity stages
viz. immature, early maturing, ripe and spent could be distinguished based on morphological characteristics of testes and are referred as stage I, II, III and IV, respectively. Similarly in female, based on ovary morphology and ova diameter six stages i.e. immature, developing virgin, early maturing, late maturing, ripe and spent were defined as stage I, II, III, IV, V and VI, respectively. For male only body muscle while in female both muscle and ovary were analysed for the proximate component analysis.

In muscle tissue of male, moisture content showed small variation during the different gonadal maturity stages (Fig. 7.6a). Protein content gradually decreased from immature stage (854.88 mg/g) to the matured stage (820.66 mg/g) followed by a significant rise in spent spawners (861.86 mg/g) (Fig. 7.6b). Lipid content was almost constant during the first two stages of maturity, but increased to 54.67 mg/g in the matured spawner. This was followed by a distinct fall (38.06 mg/g) in the spent spawners (Fig. 7.6c). Carbohydrate content was maximum (18.49 mg/g) in the matured spawners whereas in other stages it was almost constant with little variation (Fig. 7.6d). Ash content was low in immature and mature spawners but high in maturing and spent spawners (Fig. 7.6e). Organic carbon was high in immature and mature but low in maturing and spent fish (Fig. 7.6f). Calorific content was high in immature specimen (5.36 K cal/g) followed by a decrease (5.18 K cal/g) in matured and then gradually increased till spent phase (5.26 K cal/g) (Fig. 7.6g).
Maturity stages (Male)

(a)

(b)

(c)

(d)

Fig. 7.6
Fig. 7-6 Variation in biochemical constituents of muscle tissue of male *E. suratensis* with respect to different gonadal maturity stages, a) Moisture b) Protein c) Lipid d) Carbohydrate e) Ash f) Organic carbon g) Calorific content.
In female muscle the moisture content did not show much variation (78.0 to 79.28%) with reference to gonadal maturity (Fig. 7.7a). Protein content gradually decreased through immature (884.36 mg/g) to ripe stage (839.25 mg/g) followed by an increase in the spent spawners (878.10 mg/g) (Fig. 7.7b). Lipid content was almost stable in the first three maturity stages, later increased gradually till ripe stage (71.35 mg/g) which was followed by a sudden decrease to the minimum in spent spawners (36.02 mg/g). This concentration was even lower than that observed for the first three stages of maturity (Fig. 7.7c). Carbohydrate content almost showed a steady state (9.22 - 12.66 mg/g) throughout the maturity cycle (Fig. 7.7d). The ash content was also stable in the first four maturity stages. Thereafter, it decreased to the minimum (46.15 mg/g) in ripe spawner and showed the highest content (59.60 mg/g) in the spent spawner (Fig. 7.7e). Organic carbon did not vary much in the first three stages of maturity and gradually increased to 490.99 mg/g in the ripe stage followed by a fall (431.88 mg/g) with a minimum concentration in the spent stage (Fig. 7.7f). Calorific content also showed similar trend up to third stage followed by an increase (5.61 K cal/g) in the late maturing stage, and later decreased to its minimum (5.39 K cal/g) in spent stage (Fig. 7.7g).

The moisture content showed wide fluctuations during the various stages of gonadal maturity, with maximum value (89.33%) in immature and the minimum value (61.28%) in ripe ovary. The moisture content decreased gradually till third stage (81.08%)
Maturity stages (Female)

Muscle

Ovary

(a)

(b)

(c)

(d)

Fig. 7.7
Fig. 7.7 Variation in biochemical constituents of muscle tissue and ovary of female E. suratensis with respect to different gonadal maturity stages, a) Moisture b) Protein c) Lipid d) Carbohydrate e) Ash f) Organic carbon g) Calorific content.
which was followed by a sudden fall (64.28%) in IV stage, afterwards the values stabilized (61.28%) in the V stage (Fig. 7.7a). The value increased to 86.09% in spent spawner which is at par with the developing virgin. The protein content was high (673.17 mg/g) in the ovary of immature virgin and then gradually decreased (573.37 mg/g) till stage III. The value increased in the late maturing stage (622.39 mg/g) and remained stable throughout spent stage (Fig. 7.7b). The protein level of ovaries was relatively less than the muscle on dry weight basis, whereas, on wet weight basis it was higher than the muscle content in late maturing and ripe stage. The lipid content was minimum in immature ovary (118.29 mg/g), which gradually increased (188.50 mg/g) till stage III and thereafter, almost remained stable till ripe stage. The lipid content in spent ovary was higher (159.43 mg/g) than the virgin maturing (140.37 mg/g) (Fig. 7.7c). The carbohydrate content in the ovary was maximum (100.28 mg/g) in immature stage and gradually decreased to the minimum (64.98 mg/g) in the ripe stage, followed by marginal increase (79.09 mg/g) in spent ovary (Fig. 7.7d). The carbohydrate content of ovaries was always higher than the body muscle. Ash content was estimated only in III, IV and V stages due to lack of sufficient sample and its value were found to be almost stable (Fig. 7.7e). The organic carbon was almost stable (616.67 - 630.82 mg/g) during the first three maturity stages, but increased (666.66 - 671.88 mg/g) in late maturing and ripe stage (Fig. 7.7f). The organic carbon decreased to 641.43 mg/g in the spent ovaries, though it was marginally higher than the first three maturity stages of ovary. The organic carbon of ovary was always higher
than the muscle values. Calorific content was stable (5.31 - 5.39 K cal/g) in first three maturity stages with some marginal variations, however, it gradually increased to its peak value (5.56 K cal/g) in the ripe ovary (Fig. 7.7g). The calorific content was minimum (5.22 K cal/g) in the spent ovary.

7.3.2 Variation due to size group: Biochemical composition was also estimated in the samples of various size groups viz. 100, 120, 140, 160 and 180 mm which corresponds to the relative age of 7 - 8, 9 - 10, 12 - 13, 16 - 17 and 23- 24 months, respectively. In both the sexes, the moisture content was high (80.4%) in 100 mm size group (Fig. 7.8a), but was almost stable (77.65 -79.10%) in other size groups. There was no distinct variations in protein content (854.88 - 873.94 mg/g) of the muscle as the fish grows in size (Fig. 7.8b). Lipid content increased in both the sexes with increase in size till 160 mm and thereafter decreased in 180 mm group and the values were on par with 100 mm size (Fig. 7.8c). The lipid content of female was either equal or less than the male. Carbohydrate content was almost stable up to 160 mm but increased to its maximum in 180 mm. The carbohydrate levels were comparatively higher in females than the males except in 100 mm size group (Fig. 7.8d). The ash content in male was observed to increase marginally (46.61 - 56.62 mg/g) with increase in size. In female it was less (51.18 mg/g) in 100 mm group, then marginally increased in 120 and 140 mm groups, but again reduced and remained stable in higher size groups (Fig. 7.8e). Overall variation in ash content was insignificant. The value of organic carbon was stable up to 120 mm group in male but gradually
Fig. 7.8
Fig. 7.8 Variation in biochemical constituents of muscle tissue of male and female *E. suratensis* with reference to different size groups/age, a) Moisture b) Protein c) Lipid d) Carbohydrate e) Ash f) Organic carbon g) Calorific content.
decreased with increase in size, though it was not so conspicuous (Fig. 7.8f). In female also it was almost stable except in 120 mm group. Similarly, calorific content did not show much variation in different size groups studied (Fig. 7.8g).

7.3.3 Seasonal Variation: Monthly variation in proximate biochemical constituents on wet weight and ash free weight basis of male and female *E. suratensis* is given in Tables 7.1 to 7.4.

Moisture: The moisture content varied from 76.2 to 79.9% and from 76.86 to 80.40% in male and female, respectively. The minimum and the maximum values were in January and August respectively. The magnitude of variation in male and female was not much different i.e. 3.70% and 3.54%, respectively. The fluctuation in moisture was similar in both the sexes (Fig. 7.9). The moisture content decreased from February to May and then increased again during south-west monsoon (June - August). Subsequently, September onwards decreasing trend was evident with little variation in November until the minimum was recorded. A second peak was observed in February. The highest percentage of moisture coincided with the south-west monsoon when the salinity was minimum in the natural habitat.

Protein: The protein content in the muscle of male varied from 818.11 to 881.57 mg/g, and in female from 801.37 to 875.66 mg/g. The magnitude of variation was 63.46 mg/g and 74.29 mg/g in male and female, respectively. The calculated protein percentage on wet weight basis varied from 17.23 to 20.18 and 16.77 to 19.55 in male and female, respectively (Tables 7.1 and 7.3). On ash free
Table 7.1 Monthly variation in proximate biochemical constituents (wet wt.) of muscle tissue in male *E. suratensis*.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Months</th>
<th>Protein (%)</th>
<th>Carbohydrate (%)</th>
<th>Lipid (%)</th>
<th>Organic Carbon (%)</th>
<th>Ash (%)</th>
</tr>
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<td>0.98</td>
<td>8.99</td>
<td>1.22</td>
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<td>2</td>
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<td>0.93</td>
<td>7.90</td>
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</tr>
<tr>
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<td>0.88</td>
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<tr>
<td>4</td>
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<td>1.20</td>
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</tr>
<tr>
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<td>0.26</td>
<td>0.97</td>
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</tr>
<tr>
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<tr>
<td>7</td>
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Table 7.2 Monthly variation in proximate biochemical constituents (ash free wt.) of muscle tissue in male *E. suratensis*.

<table>
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<tr>
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<th>Lipid (%)</th>
<th>Organic carbon (%)</th>
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Table 7.3 Monthly variation in proximate biochemical constituents (wet wt.) of muscle tissue in female *E. suratensis*.

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<td>1.09</td>
<td>9.40</td>
<td>1.04</td>
</tr>
<tr>
<td>10</td>
<td>Nov.</td>
<td>17.97</td>
<td>0.27</td>
<td>1.12</td>
<td>9.42</td>
<td>1.13</td>
</tr>
<tr>
<td>11</td>
<td>Dec.</td>
<td>17.80</td>
<td>0.27</td>
<td>1.15</td>
<td>9.65</td>
<td>1.16</td>
</tr>
<tr>
<td>12</td>
<td>Jan.</td>
<td>18.54</td>
<td>0.30</td>
<td>1.87</td>
<td>11.07</td>
<td>1.15</td>
</tr>
<tr>
<td>13</td>
<td>Feb.</td>
<td>17.79</td>
<td>0.27</td>
<td>0.93</td>
<td>9.04</td>
<td>1.16</td>
</tr>
<tr>
<td>14</td>
<td>Mar.</td>
<td>18.48</td>
<td>0.29</td>
<td>1.05</td>
<td>9.49</td>
<td>1.22</td>
</tr>
<tr>
<td>15</td>
<td>Apr.</td>
<td>18.51</td>
<td>0.27</td>
<td>0.76</td>
<td>9.17</td>
<td>1.42</td>
</tr>
<tr>
<td>16</td>
<td>May</td>
<td>18.24</td>
<td>0.30</td>
<td>1.08</td>
<td>9.84</td>
<td>1.19</td>
</tr>
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</table>
Table 7.4 Monthly variation in proximate biochemical constituents (ash free wt.) of muscle tissue in female *E. suratensis*.

<table>
<thead>
<tr>
<th>Sr.</th>
<th>Months</th>
<th>Protein (%)</th>
<th>Carbohydrate (%)</th>
<th>Lipid (%)</th>
<th>Organic carbon (%)</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>Feb.</td>
<td>87.93</td>
<td>2.04</td>
<td>4.36</td>
<td>46.93</td>
</tr>
<tr>
<td>2</td>
<td>Mar.</td>
<td>89.45</td>
<td>1.42</td>
<td>5.05</td>
<td>46.16</td>
</tr>
<tr>
<td>3</td>
<td>Apr.</td>
<td>90.96</td>
<td>1.61</td>
<td>4.06</td>
<td>46.05</td>
</tr>
<tr>
<td>4</td>
<td>May</td>
<td>91.17</td>
<td>1.42</td>
<td>4.97</td>
<td>47.03</td>
</tr>
<tr>
<td>5</td>
<td>Jun.</td>
<td>92.08</td>
<td>1.51</td>
<td>5.13</td>
<td>44.67</td>
</tr>
<tr>
<td>6</td>
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<td>87.86</td>
<td>1.37</td>
<td>4.72</td>
<td>47.70</td>
</tr>
<tr>
<td>7</td>
<td>Aug.</td>
<td>91.34</td>
<td>1.14</td>
<td>4.16</td>
<td>46.17</td>
</tr>
<tr>
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<td>1.37</td>
<td>4.72</td>
<td>46.61</td>
</tr>
<tr>
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<td>1.23</td>
<td>5.40</td>
<td>46.57</td>
</tr>
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<td>5.72</td>
<td>47.82</td>
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<tr>
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<td>1.37</td>
<td>8.52</td>
<td>50.39</td>
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<tr>
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<td>1.42</td>
<td>4.79</td>
<td>46.43</td>
</tr>
<tr>
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<td>1.46</td>
<td>5.28</td>
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</tr>
<tr>
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<td>1.37</td>
<td>3.79</td>
<td>45.71</td>
</tr>
<tr>
<td>16</td>
<td>May</td>
<td>88.94</td>
<td>1.46</td>
<td>5.30</td>
<td>48.00</td>
</tr>
</tbody>
</table>
Fig. 7.9 Monthly variation in moisture of muscle tissue of *E. suratensis*.

Fig. 7.10 Monthly variation in protein content of muscle tissue of *E. suratensis*.
weight basis, the protein content in male varied from 86.22 to 93.29% and in female 84.36 to 92.46% (Tables 7.2 and 7.4). The protein content decreased marginally (873.94 - 871.97 mg/g) from February to May in male, but fell significantly in June (818.11 mg/g). The values increased gradually till September (881.57 mg/g). From October onwards the protein content showed decreasing trend till January (847.88 mg/g) and then suddenly increased in February (875.06 mg/g) (Fig. 7.10). In female, there was no distinct pattern except wide fluctuation during June to October (823.98 - 875.66 mg/g) and showed a gradual decrease from November to January (852.04 - 801.37 mg/g). It suddenly increased in February (862.36 mg/g), with little fluctuation till May.

**Lipid:** Lipid content in male varied from 33.14 - 58.43 mg/g, 3.48 - 6.14% and 0.72 - 1.25% on dry weight, ash free weight and wet weight basis, respectively. There was no distinct pattern, however, the lipid content increased in harmonic progression till July (57.78 mg/g) and then gradually decreased till September (40.64 mg/g) followed by harmonic progression till December (58.43 mg/g). January onwards it started decreasing till March (33.14 mg/g) and then remained almost stable (Fig. 7.11). However, two distinct peaks (July and December) and troughs (March, April, May) could be distinguished.

In female, the lipid content fluctuated widely from 35.47 - 80.95 mg/g, 3.79 - 8.52%, 0.76 - 1.87% on dry weight, ash free dry weight and wet weight basis, respectively. It showed a distinct seasonal pattern with fluctuation from February to April (38.52 - 47.65 mg/g) followed by a gradual increase till
Lipid (mg/g)

<table>
<thead>
<tr>
<th>Month</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
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<tr>
<td>A</td>
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<td>M</td>
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<td></td>
</tr>
</tbody>
</table>

Fig. 7.11 Monthly variation in lipid content of muscle tissue of E. suratensis.

Carbohydrates (mg/g)

<table>
<thead>
<tr>
<th>Month</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
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<td>M</td>
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<td>A</td>
<td></td>
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<tr>
<td>M</td>
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<td></td>
</tr>
</tbody>
</table>

Fig. 7.12 Monthly variation in carbohydrate content of muscle tissue of E. suratensis.
June (48.34 mg/g). The lipid content decreased gradually till August (38.47 mg/g) and then went on increasing up to January when the maximum value (80.95 mg/g) was observed. Thereafter, it again decreased in pulses till May. Relatively, lower values were recorded during April and August.

**Carbohydrate:** Carbohydrate content in male varied from 8.17 - 13.54 mg/g, 0.86 - 1.44%, 0.16 - 0.31% on dry weight, ash free dry weight and wet weight basis, respectively. Similarly, in females it varied from 10.88 - 19.39 mg/g, 1.14 - 2.04%, 0.21 - 0.40%, respectively, on dry weight, ash free weight and wet weight basis. In male, it followed an almost constant trend from February to April (12.93 - 13.54 mg/g) and then marginally decreased in May. The values remained stable during June till October (11.43 - 11.99 mg/g). There was a sharp decrease in November (8.17 mg/g) followed by gradual increase till February (13.58 mg/g). Further decrease in March (10.26 mg/g) was followed by almost constant level till May.

In female the carbohydrate content was high from February to July (12.98 - 19.39 mg/g) and low during August to October (10.88 - 11.74 mg/g) followed by high values (12.66 - 13.88 mg/g) from November to May (Fig. 7.12).

**Ash:** Ash content in male varied from 47.27 - 60.87 mg/g, 1.05 - 1.32% on dry weight and wet weight basis, respectively. The ash content followed a harmonic pattern of peaks and troughs with minimum in December and January, and maximum in March and April. In female, it varied from 50.07 - 66.15 mg/g, 1.00 - 1.42%,
respectively on dry and wet weight basis. The seasonal pattern is almost similar in both of the sexes (Fig. 7.13) with minimum being in January and the maximum in April.

**Organic carbon**: Organic carbon in male varied from 381.16 - 470.94 mg/g, 7.09 - 11.20% on dry and wet weight basis, respectively (Fig. 7.14 and Table 7.1). Organic carbon is almost stable between May to November followed by a peak in January (470.94 mg/g) and the trough in March April (413.43 - 393.29 mg/g). In female, the organic carbon ranged from 420.90 to 478.71 mg/g, 8.58 - 11.07% on dry and wet weight basis respectively. The organic carbon trend was almost constant with a peak in January (478.71 mg/g) and a trough in February to April.

**Calorific content** - Calculated calorific content in male varied from 5.11 - 5.54 K cal/g, 5.38 - 5.84 K cal/g and 1.07 - 1.27 K cal/g on dry, ash free and wet weight basis, respectively. There was no specific seasonal trend except a probable error in June. In female the calorific content varied from 5.13 - 5.51 K cal/g, 5.42 - 5.82 K cal/g and 1.04 - 1.24 K cal/g on dry, ash free and wet weight basis, respectively. The high values were recorded during May-June, October-November, January-February and low during July to September (Fig. 7.15).

### 7.4 DISCUSSION

The protein and moisture content of the muscle tissue
Fig. 7.13 Monthly variation in ash content of muscle tissue of *E. suratensis*.

Fig. 7.14 Monthly variation in organic carbon of muscle tissue of *E. suratensis*.
Fig. 7.15 Monthly variation in calorific content of muscle tissue of *E. suratensis*
gradually decreased as the gonadal maturity in female approached the ripe and mature stage in male which corresponds to the cessation of feeding in the ripe and mature stage, respectively. It has been reported that lean fish upon starving (Groves, 1970) mobilize the body protein as an endogenous source of energy. Thus, the decrease in protein level in both the sexes could be compared to the starving condition (Groves, 1970). Salmonids have been reported to mobilize protein from the muscle in response to low feed intake during spawning migration (Mommsen et al., 1980; Konagaya, 1982). A drop in muscle protein of Oncorhynchus mykiss in mature fish was associated with decreased feed availability and prolonged physical activity (Kiessling et al., 1991). Thus the present observation corroborates earlier findings.

In contrast, the lipid levels were high in late maturing and ripe fish in females and mature males. The increase in rainbow trout muscle fat was reported in immature fish and the maturing males, but no increase in female towards the spawning. This indicates that maturing female spent maximum energy in the development of gonad in the later part of maturation, thus less surplus energy is deposited as muscle fat. It appears that the fish rely on the energy reserves from the body and viscera during the period which results in decreased body fat. Male, as compared to female does not produce sex products in large quantity and as a consequence, sexual maturation seems to be less energy demanding than the female, thus enabling them to store surplus energy as fat in the musculature (Tveranger, 1985).
Male and female of Sockeye salmon (*Oncorhynchus nerka*) used fat reserves for spawning migration. Female gonads grew much faster than males, as a consequence, female spent more energy on gonadal growth (Idler and Bitners, 1959). The females therefore, use higher amount of fat and protein than males. In northern pike (*Esox lucius*), the food uptake reduced before and during spawning and the fish had to use endogenous energy reserve for gonadal growth. The liver is used as a storage house of large amount of lipid, which are used for maturation of gonad and spawning activity (Janagaard, 1967).

Feeding activity ceases during spawning in *E. suratensis* and after the hatching of larvae the parents are involved in brood care. Thus, for a considerable period there is no regular feeding by the fish. In present study, although male do not spend much energy during the maturation of gonad as compared to female, they indeed equally participate in pairing, courtship, selection of spawning site, nest building and finally the brood care. Thus, the exogenous energy deficit period is prolonged unlike other reported species, which might be influencing intensive feeding activity during the post spawning period. The average lipid content of *E. suratensis* is about 5% and thus it can be classified as a lean fish. Such fishes upon starvation mobilize body protein as an endogenous source of energy. Therefore, *E. suratensis* may be utilising the body protein, and the lipid stored around the coiled intestine (Plate 7.1) during the initial phase of cessation of feeding. Since the former was found to
decrease gradually as the gonadal maturity approached mature stage and the later were never observed in the ripe and spent fish. In *Oreochromis niloticus*, excess energy derived from the food is deposited as fat in both carcass and viscera, and the later act as a "sink" for fat storing deposits long after the flesh has been loaded to capacity (Hanley, 1991). Therefore, the visceral lipid may be utilised earlier than the muscle. Being the accessory fat, early utilisation provide space in the visceral cavity for the growing ovary and digestive tract to accommodate consumed food. The present findings of higher level of lipid in the muscle of ripe stage corroborates the earlier findings (Bruce, 1924; Chanon and Saby, 1932; Apparao, 1967). Thus, in *E. suratensis*, the protein and visceral lipid reserve may be utilised in the pre-spawning period and the muscle reserve in the post-spawning period. Therefore, the sequence of mobilisation of endogenous source of energy could be the possible reason of high level of lipid content in the mature spawners.

The marginal decrease in ash content and the simultaneous increase in organic carbon in ripe stage could be attributed to the elevated levels of lipid, since the latter displayed linear relationship with the former \( r = 0.8739; n = 6; p < 0.05; \text{ Table 7.5} \). The calorific content did not show much variation in female, but decreased in ripe spawner in comparison to late maturing stage. This indicates that the fish cease to feed before attaining ripe stage and starts utilising endogenous source of energy. This continues till the spent stage when the minimum value of calorific content is recorded.
Table 7.5 Correlation matrix of biochemical constituents in muscle tissue of female in different gonadal maturity stages. (n=6)

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
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<td>Moist.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prot.</td>
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<td>Carb.</td>
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<tr>
<td>Lip.</td>
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<td>-0.8511</td>
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<td></td>
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<tr>
<td>Ash</td>
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<td>0.8223</td>
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<td>-0.7805</td>
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<tr>
<td>Org.C.</td>
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<td>-0.9894</td>
<td>0.5158</td>
<td>0.8739</td>
<td>-0.8924</td>
<td>1.0000</td>
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</tr>
<tr>
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<td>-0.2295</td>
<td>-0.1173</td>
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<td>0.0149</td>
<td>0.2061</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

DF = 4

Table 7.6 Correlation matrix of biochemical constituents in female gonads at different gonadal maturity stages. (n=6).

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</tr>
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<td>Lip.</td>
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<td>-0.9561</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1.0000</td>
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</tr>
<tr>
<td>Cal.Con.</td>
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<td>0.3790</td>
<td>-0.4415</td>
<td>0.2620</td>
<td>0.7212</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

DF = 4

Significant at 0.1 = 0.7293
0.05 = 0.8114
0.02 = 0.8822
0.01 = 0.9172
0.001 = 0.9740

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The moisture content was the lowest in the highest class group (180 mm.). There was no variation in protein level as the fish grew in size or age. The lipid levels were low in the 100 mm class, since most of the energy was diverted towards growth. The lipid level increased in higher class (120-160 mm), and then decreased in the largest class. The increased level at 120-140 mm size coincide with the first maturity while the decreased level in the largest class could be attributed to the gradual deteriorating condition of the spawners. *E. suratensis* is a continuous spawner, and may not be getting sufficient food and time for restoration of energy between the successive spawnings, at least, in Goa waters. There appears to be no distinct change in the ash, organic carbon and calorific content with respect to fish size and age.

There was a progressive decrease in moisture and carbohydrate content of ovary and analogous increase in lipid as the fish approached ripe stage. However, during the spent phase the lipid content fell significantly, and the moisture and carbohydrate levels shot up. The moisture content was positively correlated with carbohydrate ($r = 0.8229; n = 6; p < 0.05$) and inversely related to organic carbon ($r = -0.866; n = 6; p < 0.05$) and calorific content ($r = -0.956; n = 6; p < 0.05$; Table 7.6). Therefore, it can be said that the concentration of carbohydrate decreased probably due to its preferential utilization in the early stages of gametogenesis, whereas, the concentration of other constituents increased as a result of decrease in moisture and associated increase in ovary size. The observed reduction in
moisture content could be attributed to the accumulation of maximum energy in minimum possible volume of ova as a yolk reserve for the early ontogenic stages. In spent stage, all the components except moisture and ash were observed to decrease and were at par with II and III stage, which also coincide with the gonadal maturity stages in terms of gonad morphology and ova size.

There are very few studies (Apparao, 1967; Medford and Mackay, 1978; Craik and Harvey, 1984; Devauchelle et al., 1988) on the biochemical composition of matured ovary or egg. Unfortunately most studies are restricted to the estimation of moisture, lipid and protein. In the present study carbohydrates were observed to be present in considerable quantity (64.98 - 100.28 mg/g). At this stage it is merely a speculation to say that carbohydrate reserve in ovary may be an energy store for the brief period of early developmental stages of egg, whereas lipid as a reserve for the post-hatching period. The role of glycogen in fish muscle has been described as energy reserve for brief emergency burst moment (Kiessling et al., 1991).

The increased levels of moisture during July-September could be attributed to low level of salinity (0.18%) resulting from heavy precipitation during south-west monsoon. Thus, in order to maintain osmotic balance, the moisture content might have increased during this period. On the contrary, the low moisture content in April-June could be attributed to higher salinity. The percentage of moisture in body muscle and ion composition of the tissue fluid are known to change according to the ambient
salinity as an adaptive measure to maintain the osmotic balance with the medium. In *Sebasticus marmoratus*, the moisture content of the blood was reported to decrease with the increase in chlorinity (Yamashita, 1967). He reported that in euryhaline fish, specific gravity of blood is positively correlated to the salinity of the environment. The moisture content in Atlantic salmon increased during its spawning migration to freshwater systems (Cowey et al., 1962). While rearing the mullet (*Mugil cephalus*) in freshwater medium similar pattern was observed (Perera and De Silva, 1978). A similar behaviour of increasing and decreasing body moisture has been reported for natural and cultured green mussel (Galtsoff, 1964; Parulekar et al., 1982).

The low percentage of moisture during January in female could be attributed to the high levels of lipid during this period. The correlation matrix ($r = -0.555; p < 0.05; \text{Table 7.7}$) revealed that the moisture and lipid were inversely related. This inverse relationship has been documented by various workers for different fish species (Idler and Bitners, 1959; Groves, 1970; Reintz, 1983; Weatherley and Gill, 1983; Tveranger, 1985; Aasgaard, 1987). In male the low level of moisture in January could be assigned to the concomitant impact of protein and lipid, since lipid alone did not exhibited any relationship. The organic carbon was observed to show inverse relationship ($r = -0.4787; p < 0.1$). Such relationship has been reported earlier (Geiger and Borgstrom, 1962). Furthermore, the low level of moisture during May–June and January also coincides with breeding seasons when matured specimens were predominant.
Table 7.7 Correlation matrix of biochemical constituents in muscle tissue of female during different months. (n=16)

<table>
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<tbody>
<tr>
<td>Moist.</td>
<td>1.0000</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Prot.</td>
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</tr>
<tr>
<td>Carb.</td>
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<td>-0.1034</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lip.</td>
<td>-0.5551</td>
<td>-0.5219</td>
<td>-0.1319</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ash</td>
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<td>0.1740</td>
<td>0.0259</td>
<td>-0.3960</td>
<td>1.0000</td>
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</tr>
<tr>
<td>Org.C.</td>
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<td>-0.6770</td>
<td>-0.0398</td>
<td>0.7937</td>
<td>-0.4984</td>
<td>1.0000</td>
<td></td>
</tr>
<tr>
<td>Cal.Con.</td>
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<td>0.6450</td>
<td>-0.1640</td>
<td>0.3123</td>
<td>-0.1624</td>
<td>-0.0436</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

DF = 14

Table 7.8 Correlation matrix of biochemical constituents in muscle tissue of male during different months. (n = 16)

<table>
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<td>Moist.</td>
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<td>-0.1479</td>
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<tr>
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<td>0.2156</td>
<td>1.0000</td>
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<tr>
<td>Ash</td>
<td>0.3436</td>
<td>-0.0621</td>
<td>0.2401</td>
<td>-0.2586</td>
<td>1.0000</td>
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</tr>
<tr>
<td>Org.C.</td>
<td>-0.4787</td>
<td>0.0018</td>
<td>0.0426</td>
<td>0.5342</td>
<td>-0.7697</td>
<td>1.0000</td>
<td></td>
</tr>
<tr>
<td>Cal.Con.</td>
<td>0.2051</td>
<td>0.7672</td>
<td>0.0586</td>
<td>0.5466</td>
<td>-0.2054</td>
<td>0.3425</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

DF = 14

Significant at 0.1 = 0.4259 0.01 = 0.6276 0.05 = 0.4973 0.001 = 0.7420 0.02 = 0.5742

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Protein content was at its low level in male during June and January and could be attributed to the breeding season, when the lipid content was comparatively high, though it showed an insignificant relationship. During non-breeding season (April-May and August), the high protein was associated with low lipid content. In female too, the low level of protein was accompanied by analogous high lipid content in January which again coincided with the breeding season. While studying body composition of rainbow trout in relation to first sexual maturity Tveranger (1985) observed that with increase in fat content the protein content is reduced with a simultaneous increase in dry matter. In female, the protein showed a significant inverse relationship with lipid \( r = -0.522; p < 0.05 \) and organic carbon \( r = -0.677; p < 0.01 \), and positive relationship with calorific content \( r = 0.645; p < 0.02 \). Similarly in male, the protein was directly related to calorific content \( r = 0.767; p < 0.001 \); Table 7.8). These observations revealed that protein content decreases with increase in the lipid content, but the increase in dry matter may not respond to increase in lipid content in brackishwater species, where the moisture content/dry matter also depends upon the density of the aquatic medium. A positive and significant relationship between calorific content and protein suggest that it is a major source of energy in \( E. \) suratensis.

In both the sexes, the lipid content was high during the breeding season (June July and December-January) and the seasonality was evident in case of female. Lipids are the depot of energy, which the fish utilises during adverse feeding
conditions. Overall variations in lipid content in the muscle was not high, since they were also observed to be stored around the coiled intestine (Plate 7.1). Hails (1983) reported the storage of lipid around the intestine in *Trichogaster pectoralis*. At spawning, the rainbow trout has been reported to retain higher amount of lipid in ovaries and gastro-intestinal tract (Washburn et al, 1990).

In male *E. suratensis*, increase in lipid was directly proportional to increase in organic carbon \( r = 0.534; p < 0.05 \) and calorific content \( r = 0.546; p < 0.05 \). Similarly, in female, the lipid was directly related to organic carbon \( r = 0.794; p < 0.001 \) but inversely related to moisture \( r = -0.555; p < 0.05 \) and protein \( r = -0.522; p < 0.05 \). Thus, in male the lipid content is independent of other components, though protein and ash do have some inverse impact on its concentration. *E. suratensis* ceases or rarely feed during breeding, since it is deeply involved in pairing, courtship and spawning followed by prolonged parental care. Thus, the lipid stored in body muscle and around the coiled intestine are used as energy reserve during spawning. This could be the possible reason of higher levels of lipids during May-July and December-January.

Carbohydrates contribute a minor part of the muscle, though its role is similar to that of lipid as the main fuel (Walton and Cowey, 1982). There is no distinct pattern in the concentration of carbohydrate, which were observed to change invariably. Carbohydrate was observed to have absolutely no
relationship with any of the other biochemical components. It has been referred that lactic acid is produced during the breakdown of glycogen, and the overall elevation in lactate level and fall in glycogen concentration in lateral muscles following exercise has been observed in various fish species (Black et al., 1961). For example, in rainbow trout a fall of 50 -88% in glycogen content in muscle after a few minutes of exercise has been observed (Miller et al., 1959; Black et al., 1962). The glycogen content was reported to fall by 50% after moderate and by 80% after high speed exercises for 30 minutes in Atlantic cod Gadus morhua (Beamish, 1968). The muscle glycogen depletion and analogous lactate build-up with increasing speed of exercise was also recorded by Broughton and Goldspink (1978) in roach (Rutilus rutilus). Hochachka (1961) reported that after 5 minutes exercise, the glycogen content in trout fell by 65 - 70% in trained fish but by only 40% in untrained, and suggested that stamina was not limited by the amount of food reserve but by the ability of blood to tolerate high levels of lactate. Perera and De silva (1978) reported that in Mugil cephalus, the major portion of carbohydrate is utilised for energy and the fraction estimated is derived from blood glucose and structural sources such as glyco-protein and glyco-lipid, indicating that it does not store carbohydrate. Carbohydrate are utilised for energy in trout, thus sparing protein for building of the body (Phillips et al., 1966).

The decrease in muscle glycogen has been reported to be associated with change in salinity from freshwater to seawater in
case of Oncorhynchus kisutch and O. mykiss (Sheridon et al., 1985; Kiessling et al., 1991). The latter author emphasised the importance of glycogen as energy store, reserved for brief emergency burst momenta. Thus, in the present case the erratic variation in carbohydrate content could be assigned to daily tidal currents, seasonal changes in salinity during monsoon and the capture stress.

Ash content varied in a narrow range (47.27 - 66.15 mg/g) and is inversely related to organic carbon in both the sexes (male, \( r = -0.768; n = 16; p < 0.001 \); female, \( r = -0.498; n = 16; p < 0.05 \)). Organic carbon represents the major total carbon of the biochemical constituents viz protein, lipid and carbohydrate, which may vary according to the conditions. The organic carbon is also high during May and January which corresponds to spawning period. This increase was associated with the increase in lipid levels, since the corresponding protein concentration is low during this period. In male, the organic carbon is positively correlated (\( r = 0.534; n = 16; p < 0.05 \)) with lipid and inversely related with the moisture (\( r = -0.4787; p < 0.1 \)) and ash (\( r = -0.7679; n = 16; p < 0.001 \)) whereas, in female, the organic carbon is directly related (\( r = 0.794; n = 16; p < 0.001 \)) with lipid and inversely (\( r = -0.677; n = 16; p < 0.01 \)) with protein and ash (\( r = -0.498; p < 0.05 \)).

In male, the calorific content was positively related with protein (\( r = 0.7672; n = 16; p < 0.001 \)) and lipid (\( r = 0.546; n = 16; p < 0.05 \)), thus the collective concentration of both the components was responsible for higher calorific content during
February and May. In female, the calorific content was directly related with protein \( r = 0.645; \ p < 0.02 \). Thus, the low calorific content during the breeding season can be attributed to decrease in protein content, which in turn is related to poor feeding conditions. In lean starving fish, body protein serves as an endogenous energy source, if body fat is present, some protein is still degraded to allow catabolic utilization of fat (Groves, 1970). Further, it has been reported that the protein increased more rapidly than water in lean growing fish. In starving fish, both water and protein are lost preferentially and protein disappears more rapidly than water.

The proximate composition of *E. suratensis*, revealed that it is rich in protein content and low in lipid. From nutritional point of view, based on biochemical analysis it can be placed in category "A" (as described by Stansby, 1962). The protein levels of *E. suratensis* are far higher than that of other reported fish species such as *Mugil cephalus* (Perera and De Silva, 1978), *Tilapia mossambica* (Pandian and Raghuraman, 1972), *Oreochromis niloticus* (Hanley, 1991), and *Chanos chanos* (Hung et al., 1980) cultivable in brackishwater environment. On the contrary it is low in lipid and perhaps is a suitable diet where protein rich and low lipid food is to be supplemented.