

7.1. Introduction

On a global scale, fish and fish products are the most important source of protein in the human diet. This protein is relatively of high digestibility compared to other protein source. It comprises of all the ten essential amino acids in desirable quantity for human consumption. All these properties brings the fish flesh to be in the same class as chicken protein and are superior to milk, beef protein and egg albumen (Srivastava, 1999).

In general, the biochemical composition of the whole body indicates the fish quality. Therefore, proximate biochemical composition of a species helps to assess its nutritional and edible value in terms of energy units compared to other species. Variation of biochemical composition of fish flesh may also occur within same species depending upon the fishing ground, fishing season, age and sex of the individual and reproductive status. The spawning cycle and food supply are the main factors responsible for this variation (Love *et al.*, 1980).

An increasing amount of evidences suggest that due to its high content of polyunsaturated fatty acid fish flesh and fish oil are beneficial in reducing the serum cholesterol (Stansby, 1985). In addition to that, the special type of fatty acid, omega-3 polyunsaturated fatty acid, recognized as an

important drug to prevent a number of coronary heart diseases (Edirisinghe, 1998). It is recommended by cardiologists to use generous quantities of fish in food to obtain adequate protein without taking in excessive fatty acids and lipids (Dyerberg, 1986; Kinsella, 1991). The nutrition experts and people who are interested in caloric content of the food for weight control also require information on biochemical composition of the diet.

Although several studies deal with the proximate composition of biochemical components of many commercially important fishes (Parulekar, 1964; Nair, 1965; Raja, 1969; Qasim, 1972; Ramaiyan *et al.*, 1976; Sivakami *et al.*, 1986; Sinha and Pal, 1990; Das and Sahu, 2001), but no work on similar lines has been carried out in *S. sihama* particularly from Goa waters. Therefore, the present study was undertaken to elucidate the dynamics of biochemical composition of muscle of *S. sihama* with reference to season and size.

7.2. Material and methods

Samples of *S. sihama* were collected from Zuari estuary (Dona Paula) during the period of January 2004 to April 2005. The specimens were properly cleaned in the laboratory and the total length, total weight, and sex were determined. Based on their total length, fishes were arranged into size groups. Gonads and general viscera were removed and preserved in 5 % formaldehyde for further study. Body muscle samples

(free from skin and scales) from four to six specimens were pooled together and used for the analysis of biochemical components every month. Similarly, a composite sample of fish representing different size groups was taken to study the dynamic of biochemical constituents in this tissue. Estimation of the moisture content was carried out by drying the pre-weighed wet samples at 60 °C until a constant weight was obtained. The difference in weight was calculated and expressed as percentage moisture content of the sample. Percentage was calculated by the following formula.

$$\text{Moisture \%} = \frac{\text{Wet weight of tissue} - \text{Dry weight of tissue}}{\text{Wet weight of tissue}} \times 100$$

The dried samples were finely powdered using mortar and pestle and stored in desiccator for further analysis.

Lipid was estimated by the method of Folch *et al.* (1957). Ten mg of dried sample was homogenized in 10 ml of chloroform-methanol mixture (2/1 v/v). The homogenate was centrifuged at 2000 rpm. The supernatant then washed with 0.9% saline solution (KCl) to remove the non-lipid contaminants and allowed to separate. The upper phase was discarded by siphoning. The lower phase was allowed to dry in an oven and the weight was taken. The lipid content was expressed as Percentage by the following formula.

$$\text{Lipid \%} = \frac{\text{Weight of lipid (mg)}}{\text{Weight of sample (mg)}} \times 100$$

Protein was estimated following the method of Lowry *et al.* (1951). To a 10 mg of sample 1 ml of 1N NaOH was added for protein extraction in water bath for 30 minutes. Thereafter, it was cooled at room temperature and neutralized with 1 ml of 1N HCL. The extracted sample was centrifuged at 2000 rpm for 10 minutes, and an aliquot of the sample (1 ml) was further diluted with distilled water (1/9 v/v). From the 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 reagent. After 30 minutes, sample absorbency was read at 750 nm using spectrophotometer. The results were expressed as percentage.

Total Carbohydrate was estimated by the phenol sulphuric acid method (Dubois *et al.*, 1956). Sample of dried tissue (10 mg) was treated with 2 ml of 80 % sulphuric acid and was allowed to digest for about 20 - 21 hours at room temperature. Two ml of 5 % phenol reagent followed by 5 ml of concentrated sulphuric acid were added to the digested sample and was allowed to cool. Absorbency was measured at 490 nm. The concentrations were expressed as percentage. All the values of biochemical components were expressed as percentage dry weight basis.

Caloric content was calculated by multiplying the concentration of various components with conversion factors 4.15, 9.4, and 5.65 for carbohydrate,

lipid and protein, respectively, (Phillips, 1969). The caloric values were expressed as calories per gram (cal/ gm) on dry weight basis. All the estimations were done in triplicate and mean values were calculated.

7.3. Results

Both, seasonal and size wise variation in biochemical components are tabulated in Table 7.1., Fig. 7.1. – 7.4. and Table 7.2., Fig. 7.5. – 7.8, respectively. Moisture forms the major component of the biochemical composition with an annual average value of 77.8 % \pm 1.033 throughout the investigation period. The seasonal variation was in the range of 76.2 - 79.6 %.The moisture content was observed to be high from January to March (79.3 % - 79.1 %) followed by gradual decline until June (76.9 %). It was steady during July - September after which moisture content increased until November followed by sharp decline to reach its minimum value in December 2004 (76.2 %) (Fig. 7.1.). Related to size of the fish, there was a slight fluctuation in moisture content. The value was low in the size group 85 – 94 mm and fluctuated within a very narrow range from the size group 95 - 104 mm to the size group 145 - 154 mm, followed by increase in the size group 155 - 164 mm. In the size group 165 - 174 mm moisture content was decreased followed by gradual increase until the

Table 7.1. Seasonal variation in the biochemical components of the muscle of *S. sihama*.

Month/compos	Moisture	Lipids		Carbohydrate		Protein		Total Caloric value
	%	%	cal/ g dry wt.	%	cal/ g dry wt.	%	cal/ g dry wt.	(cal/ g)
Jan	79.32	3.20	300.80	0.46	19.09	19.21	1085.33	1405.22
Feb	79.27	2.25	211.50	0.58	24.07	19.13	1080.66	1316.23
Mar	79.08	2.65	241.27	0.52	21.58	22.80	1288.28	1551.13
Apr	78.47	3.05	286.70	0.57	23.66	23.19	1310.44	1620.80
May	77.91	4.19	393.86	0.35	14.48	22.38	1264.37	1672.71
Jun	76.99	4.07	378.74	0.54	22.53	24.73	1397.34	1798.61
Jul	77.04	3.79	356.73	0.44	18.47	21.65	1222.96	1598.16
Aug	78.09	3.12	292.97	0.56	21.08	22.63	1278.36	1592.41
Sep	78.20	2.94	276.26	0.63	26.23	23.89	1349.51	1652.00
Oct	78.70	2.69	249.53	0.54	22.41	24.27	1371.09	1643.03
Nov	79.40	2.16	202.10	0.48	20.09	24.96	1410.17	1632.35
Dec	76.20	3.04	286.23	0.44	18.43	21.23	1199.63	1504.29
Jan	77.43	3.00	246.75	0.53	19.30	22.67	1280.70	1546.74
Feb	77.06	3.76	353.84	0.58	24.07	22.97	1297.61	1675.52
Mar	76.62	3.87	363.47	0.62	25.65	20.46	1155.89	1545.01
Apr	76.70	2.83	280.12	0.59	24.61	23.73	1340.77	1645.50

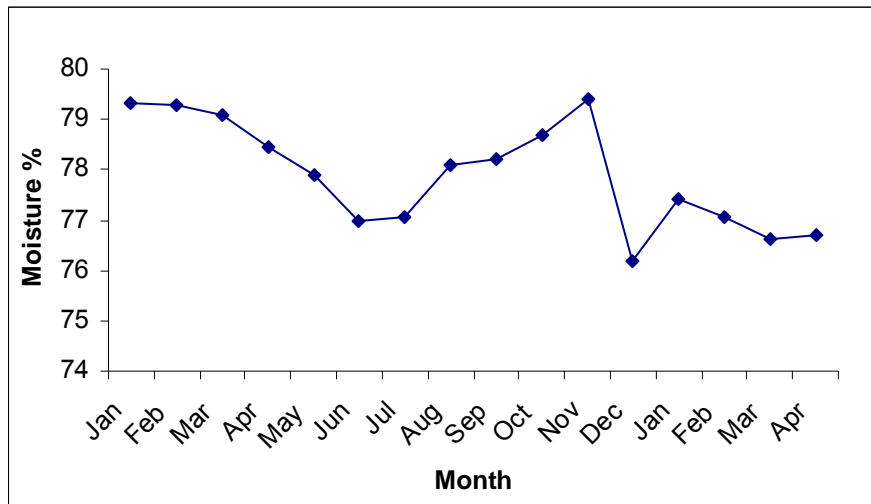


Fig. 7.1. Monthly variation of Moisture content in *S. sihama*.

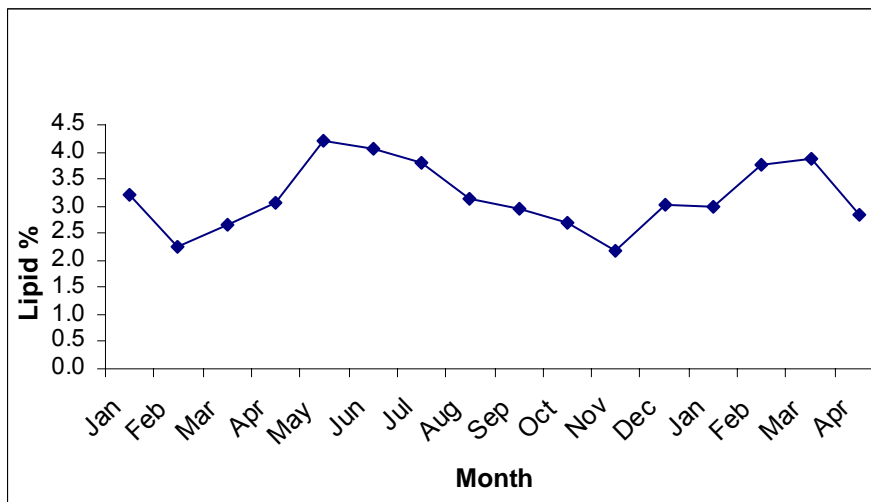


Fig. 7.2. Monthly variation of Lipid content in *S. sihama*.

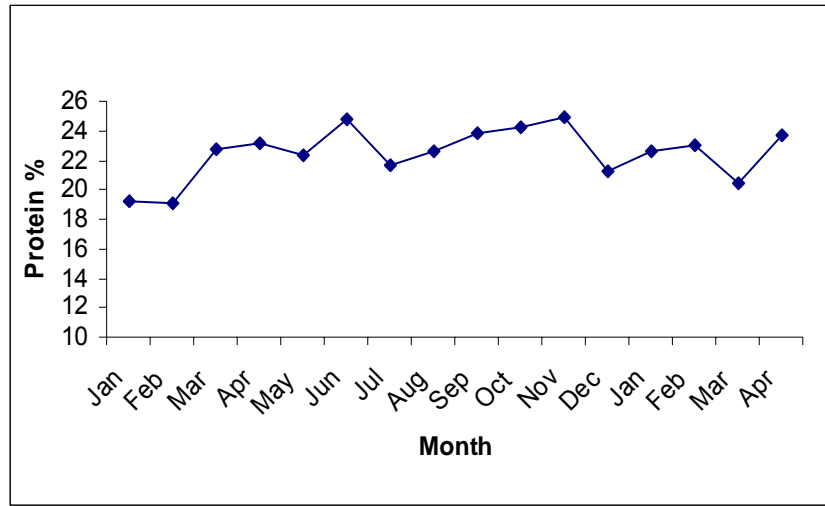


Fig. 7.3. Monthly variation of protein content in *S. sihama*.

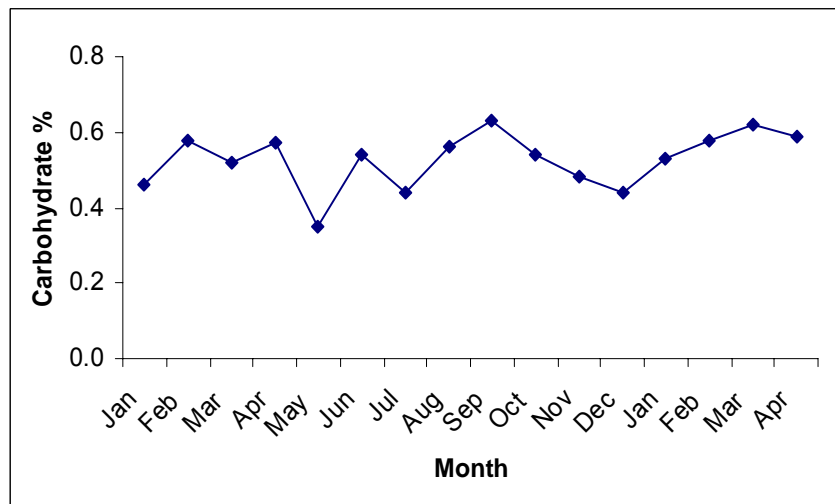


Fig. 7.4. Monthly variation of carbohydrate content in *S. sihama*.

size group 205 - 214 mm. There after the moisture content was decreased (Fig. 7.5.). The moisture content was independent of size class as no coincident pattern was noticed.

Seasonal variation of Lipid content ranged between 4.2 % in August and 2.2 % in November (Fig. 7.2.). The average value was about 3.2 % \pm 0.615 indicating that this species is moderate fat fish. The percentage of lipid content showed a low values during January - April, followed by sharp increase in May - June just prior to spawning season. Thereafter, gradual decline was observed to reach its lowest value in November, after which the value increased. Lowest value coincided with peak spawning season. Inverse relationship between lipid and moisture content was observed from the negative correlation between the two constituents ($r = - 0.6$, $P < 0.05$). Lipid content in relation to size group showed a slight fluctuation (Fig. 7.6.). High value was recorded in the size group 85 – 94 mm followed by decrease in the next size group, 95 -104 mm. Thereafter, gradual increase in lipid content was observed until the size group 145 - 154 mm. Sharp decrease was observed in the size group 155 - 164 mm, which coincided with the first maturity, followed by increase in the next size group 165 – 174 mm. Thereafter, lipid content decreased and showed low values in 205 – 214 mm and 225 - 234mm size groups. No consistent pattern was discernible in relation to the size of the fish.

Table 7.2. Changes in the biochemical components of the muscle of *S. sihama* in different size groups.

Size group/ Composition	Moisture %	Lipids		Carbohydrate		Protein		Caloric value Total cal/ g
		%	cal/ g dry wt	%	cal/ g dry wt	%	cal/ g dry wt	
85-94	75.95	3.75	352.50	0.71	29.40	17.78	1004.85	1386.75
95-104	77.07	2.99	262.26	0.54	22.59	13.84	782.07	1066.91
105-114	77.54	3.24	310.72	0.50	20.94	16.98	959.36	1291.01
115-124	77.32	3.25	305.97	0.53	21.81	16.96	958.19	1285.97
125-134	77.57	3.01	283.96	0.51	21.12	14.45	816.47	1121.55
135-144	77.46	3.89	347.92	0.53	20.25	17.58	993.18	1361.35
145-154	77.21	3.46	323.22	0.49	20.50	21.02	1187.39	1531.10
155-164	77.88	3.11	298.06	0.49	20.52	13.09	739.49	1058.07
165-174	77.07	3.70	333.23	0.52	21.56	17.96	1014.76	1369.55
175-184	77.80	2.99	279.31	0.53	21.85	14.96	845.05	1146.21
185-194	77.63	2.93	275.42	0.48	20.00	16.11	910.37	1205.79
195-204	77.80	2.55	239.70	0.40	16.76	16.02	905.12	1161.58
205-214	78.38	2.25	211.50	0.46	19.21	18.50	1045.09	1275.79
215-224	77.71	2.65	249.10	0.38	15.85	15.31	864.88	1129.83
225-234	77.24	1.60	150.40	0.43	17.97	18.15	1025.26	1193.63

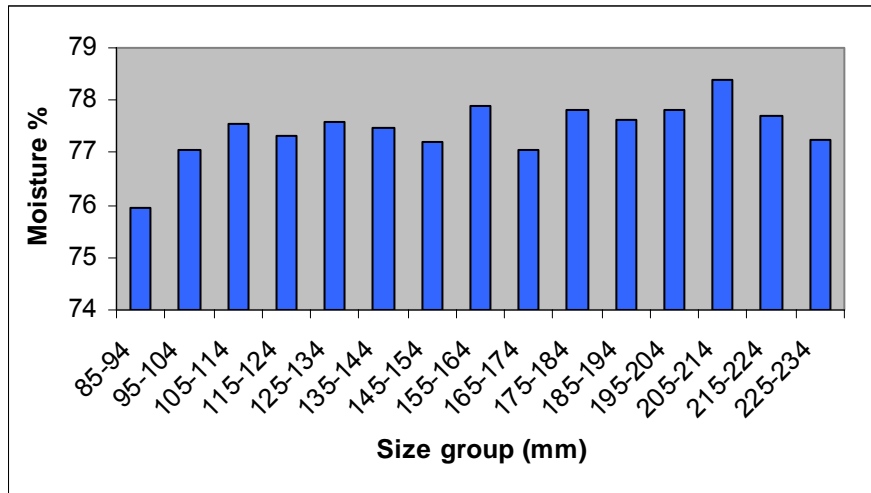


Fig. 7.5. Moisture content in different size groups of *S. sihama*.

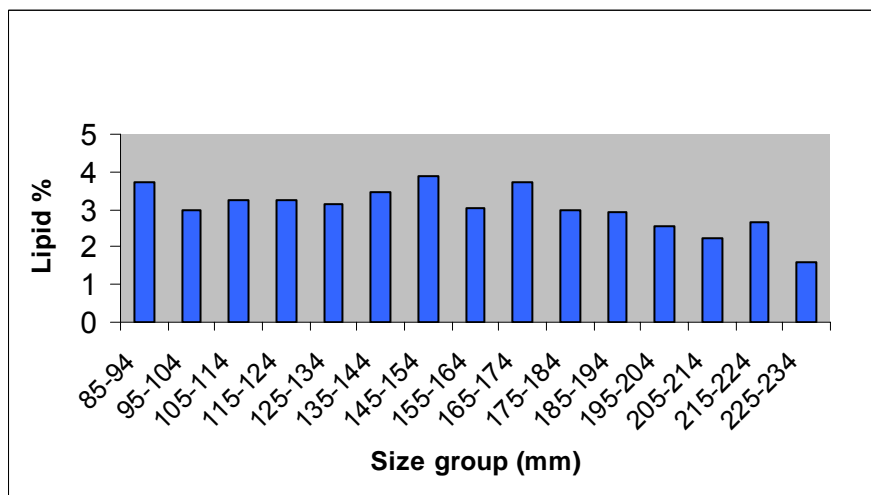


Fig. 7.6. Lipid content in different size groups of *S. sihama*.

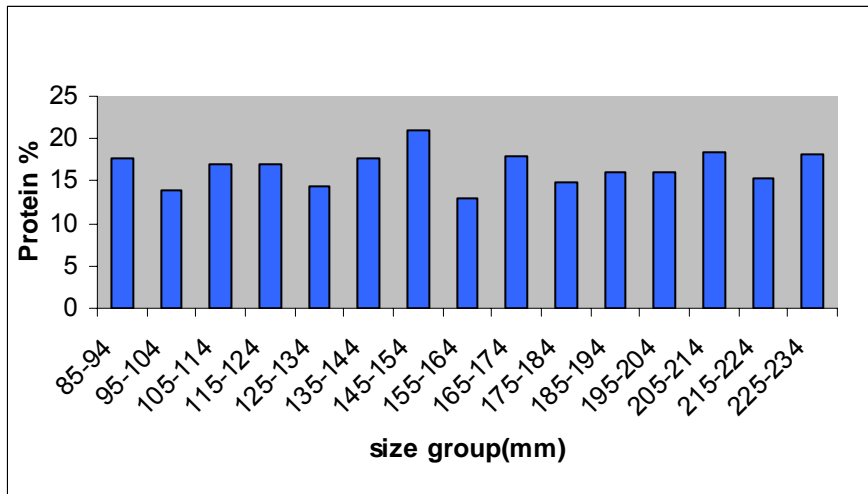


Fig. 7.7. Protein content in different size groups of *S. sihama*.

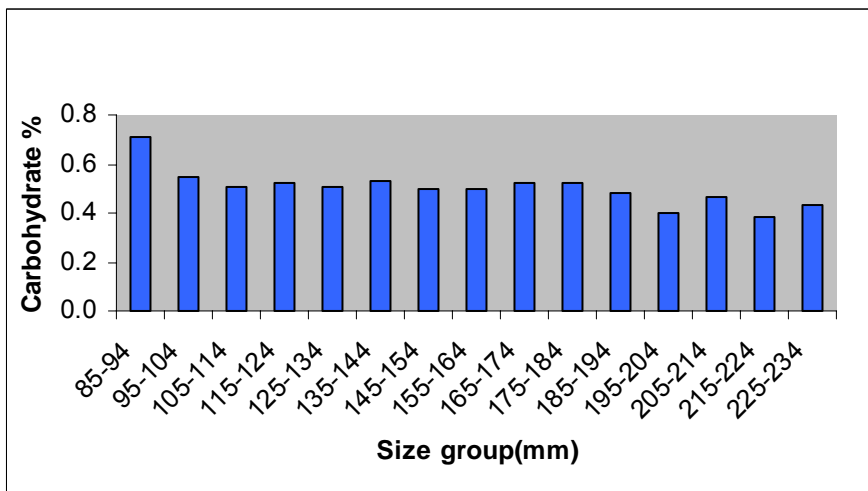


Fig. 7.8. Carbohydrate in different size groups of *S. sihama*.

As shown in Table 7.2. & Fig. 7.3., protein content of *S. sihama* varied from 19.13 to 24.96 %. The average value was calculated to be 22.49 % \pm 1.772. There was a gradual increase from January to June followed by slight decrease in July. From August to November, protein values were obviously increased to reach the maximum value (24.96 %) in November. Another decline was recorded in December - February. The lowest value was recorded in February while the highest was in November. Protein content was fluctuating with the growth in size of the fish and did not show a distinct pattern. The highest value was recorded in the size group 145 – 154 mm and the lowest value in the size group 155 - 164 mm (Fig. 7.7.).

Carbohydrate occurs in a very minute quantity in the fish tissues. In the present study, it varies between 0.35 % and 0.63 % with an average value of 0.53 % \pm 0.075. Monthly variation shown that carbohydrate fluctuated between 0.46 and 0.57 % from January to April followed by a sudden fall to 0.35 % in May. Abrupt increase was observed in June (0.54 %) to September (0.63 %) with slight decrease in July. Thereafter, there was a decline in carbohydrate values until December (0.44 %). The lowest value was recorded in May 2004 and the highest in September 2004 (Fig. 7.4.). Percentage of carbohydrate with reference to size group was stable and did not show a specific pattern (Fig. 7.8.). The results may therefore summarized that there was a similarity, observed between the carbohydrate content and protein content, in which both the constituents

have lower values synchronized with the post-spawning and early maturation phases.

Average total caloric value of the biochemical components that utilized as energy sources *viz* Lipids, protein and carbohydrates were calculated as 295.65 ± 59.09 cal/ g, 1270.82 ± 100.16 cal/ g and 21.61 ± 3.16 cal/ g on dry weight basis, respectively. Monthly variation of total caloric values is given in Fig. 7.9. Total caloric value observed during the study period ranged between 1316.23 cal/ g and 1798.61 cal/ g. Caloric value increased from February (lowest value) to June (highest values) (Fig. 7.9.). It remained stable from July to November with slight decrease in December followed by gradual increase. Total caloric value in different size group is shown in Fig. 7.10. Positive correlation was observed between the caloric value and the lipid content ($r = 0.456$, $P < 0.05$). The caloric value showed highly significant correlation with the protein content ($r = 0.856$, $P < 0.001$) (Table 7.3.).

7.4. Discussion

The chemical composition of marine organisms comes quite close to that of land animals. The principle constituents are water (66 – 84 %), protein (15 – 24 %), lipids (0.1 – 22 %), minerals (0.8 – 2 %) and sugar in very minute quantity (0.3%) at maximum value in fishes (Jacquot, 1961).

Table7. 3. Correlation matrix between the biochemical constituents and caloric content.

	<i>%Moisture</i>	<i>% Lipid</i>	<i>% Carbohydrate</i>	<i>% Protein</i>	<i>Total cal / g</i>
<i>%Moisture</i>	1				
<i>% Lipid</i>	-0.6**	1			
<i>% Carbohydrate</i>	-0.04922	-0.2493	1		
<i>% Protein</i>	-0.05258	-0.05957	0.135462	1	
<i>Total cal/ g</i>	-0.36046	0.455916	0.02469	0.85592***	1

** P < 0.01

*** P < 0.001

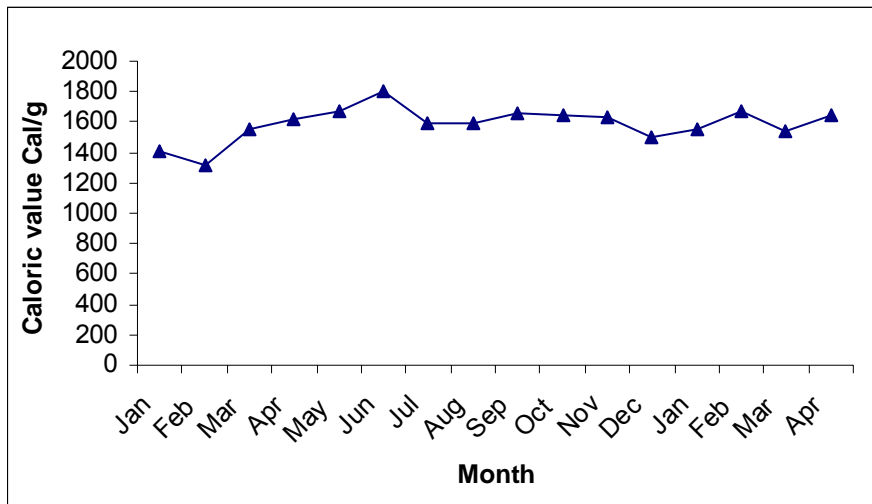


Fig. 7.9. Monthly variation in total caloric content.

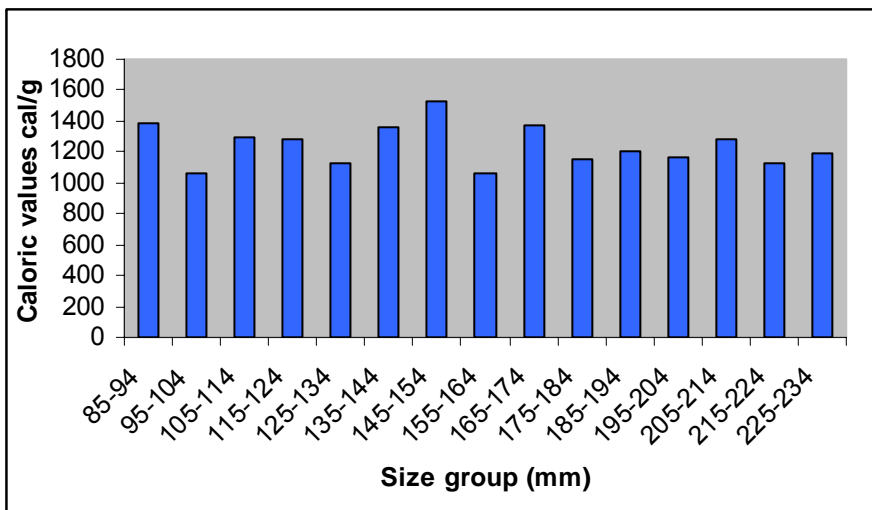


Fig. 7.10. Total caloric value in different size groups.

In the present investigation, the main constituents of the muscles of *S. sihama* were estimated to be 76.19 - 79.57 %, 19.13 - 24.96 %, 2.16 - 4.19 % and 0.3 - 0.6 % for moisture content, protein, lipids and carbohydrates, respectively. Depending upon the level of lipids in the fish muscles, fishes are classified into three categories e.g. fat fish with more than 8 % average fat content, average fat fish with fat content vary between 1 % and 8 % and lean fish with fat content less than 1 % (Srivastava, 1999). Accordingly, *S. sihama* having an average 3.16 % fat content falls into the second category (average fat fish).

The significance of the seasonal variation in biochemical constituents is complex and it is almost impossible to distinguish between the effects of many factors, which influence the biochemistry of the fish. In fishes, the biochemical content is related to maturation of gonads and the food supply (Jacquot, 1961; Medford and Mackay, 1978). The biochemical constituents are also influenced by metabolism mobility of the fish and geographical area (Stansby, 1962). Marked changes were observed in the biochemical composition of the muscle of *S. sihama* during different seasons of the year, which may be the result of the processes mentioned above.

Seasonal variation in the moisture content of the body muscles of *S. sihama* showed increase in the moisture content from January - May which coincided with the increase in water salinity. However, with the

onset of monsoon, the moisture content values increased from July until October, with the advancement in maturation and spawning activity.

The moisture was inversely related to lipid content in the present study. The inverse relationship has also been reported in marine fishes such as *Mugil cephalus* (Das, 1978); *Sarda sarda* (Zaboukas, 2006) and freshwater fishes *Mystus seenghala* (Jafri, 1968) and *Ophicephalus punctatus* (Jafri and Khawaja, 1968). Ramaiyan *et al.* (1978) reported that generally when oil content is high the moisture content is low in *Septipinna taty*, whereas, Shekhar *et al.* (2004) reported that moisture content did not significantly differ according to season in *Labeo rohita*, and was low when other constituents (Lipid, protein and carbohydrate) were high. The moisture content was high during monsoon and part of post-monsoon when the lipid content was low. That can be ascribed to similar rapid fall in lipid content during spawning season.

It has been reported by Das (1978) that high values of moisture content in *Mugil cephalus* could be due to the decline in food intake during the monsoon months when the water would be highly turbid and heavily silt loaded. Similarly, in the present study high moisture was reported during the second half of monsoon season. The results of the present study revealed that changes in moisture content in the muscle of *S. sihama* could be attributed to changes in lipid level directly and to spawning and feeding intensity indirectly.

Fat have a special importance to the animal body, which are primarily a source of energy in the diet. They offer higher calories percentage energy (9.5) than that offer by protein and carbohydrate (4 each). Fats also pad to keep different body organs in place. Moreover, fats give the diet its particular flavors.

Seasonal differences in the availability of food and changes in the reproduction cycle have considerable effect on the tissue biochemistry of the fish, particularly fat (Bumb, 1992). Fishes like other animals, store fat in their muscle for the supply of energy during starvation and reproductive phases. The greatest concentrations of fat may be found at the end of prolific feeding in summer and the least in winter (Love, 1980). Intensive feeding in *Ambassis commersoni* coincides with the occurrence of high fat content in the muscle of fish (Bumb, 1992).

Intensive feeding in *S. sihama* in January and April - May (pre-spawning) coincided with relatively high values of lipids. With the commencing of spawning season in June, feeding activity also decreased until October. There was a parallel decrease in the muscle lipid during same period. This could be attributed to less food intake. Richard (1997) suggested that during starvation the source of energy is lipid and carbohydrate in fishes.

A rise in the fat content of mackerel muscles before spawning followed by a fall after spawning was observed by Chidambaram *et al.* (1952). Reduction in the lipid content during the spawning season has been

recorded in *Bregmaceros mclellandi* (Parulekar and Bal, 1969), *Mugil cephalus* (Das, 1978) and *Ambassis commersoni* (Bumb, 1992). The fat content of common carp reached the lowest level in the spent stage, indicating its utilization in spawning activity (Masurkar and Pai, 1979).

Changes in the muscle fat were not well marked with the growth of the fish. The lipid content showed a gradual increase with the increase in length until the size group 145 - 154 mm, which showed the maximum lipid content value. Sudden decline in the next group 155 - 164 mm may correspond to first maturity. While, low values in very large sized indicates that beyond a certain size range the fish becomes leaner. This agrees with the findings of Sekharan (1950). Such distribution of lipids in the muscles of *S. sihama* suggests that the energy demands during the growth are not met by the muscle fat. Higher fat and protein were observed in ripe and gravid fish where a low level of fat and protein was recorded in spent and young fish (Bhuyan *et al.*, 2003).

Protein was the most dominant biochemical constituent in the muscle of *S. sihama*. Protein values were observed to increase from July to November when maximum numbers of mature fishes were found. Decline in protein content in December and remain almost low until February, coincided with post-spawning period. Parulekar (1964) reported maximum protein content in the spawning specimens and the minimum associated with the spent and early maturation phases. Protein content can be

correlated with the phases of maturity and spawning (Parulekar and Bal, 1969). Accordingly, protein content goes on increasing with the advancement in maturity. Similar elucidation in *Mugil cephalus* was suggested by Das (1978). He noticed that high values of protein coincided with the spawning season when the gonads were ripe and the decline coincided with post spawning period. In the present study, it seems that fish was utilizing fat as a main source of energy, sparing protein for bodybuilding. This was corroborated by findings recorded by Phillips *et al.* (1966). They found that carbohydrates were utilized for energy by trout and thus spared protein for protein purposes in the body. Protein is not an efficient energy source for fish. It will be used for energy if the available energy from other sources (lipid and carbohydrates) is insufficient (Phillips, 1969). Rattan (1994) suggested that protein and visceral lipid resources may be utilize in the pre-spawning period and the muscle reserve in the post - spawning period in *Etroplus suratensis*. Therefore, the sequence of mobilization of endogenous source of energy could be the possible reason of high level of lipid content in the mature spawners.

Van Bohemen and Lambert (1980) that the highest protein content in pre-spawning stage might be due to its ready supply suggested it by the liver. Medford and Mackay (1978) showed that muscle protein and lipid of northern pike, *Esox lucius* were high before spawning and low after spawning. This could be attributed to the fact that these constituent might have been utilized for spawning and gonadal development. While studying

the biochemical composition of *Cyprinus carpio* Sivakami *et al.* (1986) had found that protein for germ building was mobilized from the muscle especially in stages I to IV of maturity. Changes in the protein content during spawning season may occur due to changes in the endocrine system that monitors supply of nutrients to gonads from all parts of body including liver and muscles (Sinha and Pal, 1990; Jyotsna *et al.*, 1995).

Protein cycle and lipid cycle of muscle in *S. sihama* were more or less inversely related. It seems that there is an alternative uses of the energy sources (lipids and protein) in *S. sihama*. Jafri (1968) also noticed such relationship in *Mystus seenghala*. The protein cycle in muscle does not seem to be influenced greatly by feeding intensity. Heavy feeding during summer (pre-monsoon) and low feeding during post-monsoon showed no corresponding decrease or increase in protein in *S. sihama*. Similar findings were reported by Bumb (1992) in *Ambassis commersoni*, in which she observed that protein does not show any relationship with intensity of feeding. Bernard (1976) reported that hepatic and extra-hepatic and glycogen stores serves for metabolic needs during food shortage, while body protein is conserved. Similarly, Norman (1980) suggested that in snakehead, protein catabolism is reducing with the onset of starvation.

Rattan (1994) reported that moisture content and protein gradually decreased in mature male and ripe female, which corresponds to the cessation of feeding in the mature and ripe stages.

Carbohydrates formed a minor percentage of the total composition of the muscle. The low values of carbohydrates recorded in the present study could be because glycogen in many marine animals does not contribute much to the reserves in the body (Jayasree *et al.*, 1994). Ramaiyan *et al.* (1976) reported similar findings in 11 species of Clupeids. Vijayakumaran (1979) stated that carbohydrate plays a minor part in energy reserves of *Ambassis gymnocephalus* and depletion due to spawning is negligible. Phillips *et al.* (1966) reported that carbohydrates are utilized for energy in trout, thus sparing protein for building of the body.

There are two types of food energy e.g. heat, which is useful for the maintenance of body temperature and free energy that used for body metabolism. However, the food energy including both the types is express as heat. The unit used to express the food energy in nutrition studies is calorie (cal) or kilogram calorie (Kcal). Variation pattern observed in caloric values indicates a direct association between lipid content and high degree of association between protein content and caloric value. The highest caloric value was recorded in the size group 145 – 154 mm when they are \geq two years old just prior to first maturity. The value decreased in the next size group 155 – 164 mm (size at first maturity). This indicated that the fish starts utilizing the endogenous source of energy for gonads building.

The study of the proximate composition of *S. sihama* revealed that it is rich in protein and average in lipid content. Variation in biochemical composition in present study seems to be governed by spawning cycle and feeding activity.