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Seasonal biochemical characterization of muscles, liver and ovary of *M. seenghala* from Vindhya region

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Abstract
With increasing population India is facing a very serious problem of food resources. However in India a large number of peoples from different society depend upon non-vegetarian fish diet. Compared to other animals fish possess relatively greater amount of muscular tissues and therefore rich source of nutrition.
In the present study seasonal variation of biochemical composition were evaluated in liver, muscles and ovary of *M. seenghala*. In liver the glucose content was found to vary considerably from season to season. Highest level of glucose was recorded in the month of January, which gradually reduces. The lowest glucose level was recorded in June and then again increases in the month of December. Muscular tissue constitutes about 40% of the body weight and it is therefore the largest single tissue component of the body, which can suddenly change its metabolism manifold depending on its state of activity. The activity of muscles is major determinant of metabolic requirements of the body and all the circulatory and other adjustment related to this. The occurrence of carbohydrates in the muscles is not constant. The highest amount of glucose in the in the ovary and its lowest level in liver can be rerecorded during the spawning phase. The lowest glucose level in the ovary and its highest level in the liver are noted in the resting /spent phase. Glycogen quantity changes with maturation cycle of the ovary. The lowest quantity of glycogen was observed in stage II and in the following state. Glycogen content reaches to its maximum in stage IV. Highest level of glucose was recorded in ovary in the month of July, which gradually reduces, and lowest recorded in January.

Keywords: *M. seenghala*, liver, kidney

1. Introduction
Fishes are considered as the most potent staple food after food grains due to its high nutritive value, easy availability and assimilation in the human body. Accordingly, the fishery science has been widely developed in countries like Japan, Canada and USA. Even in India we have potential resources for its development, but they are not economically exploited. One of the most important aspects of this development is to increase the production to highly nutritive fish at low costs. In order to achieve this goal, culture of various fishes have been adopted after analyzing their food, feeding habit, growth pattern, reproductive capacity and various biochemical aspects. The cat fish *M. seenghala* is found is all over the India in rivers, tributaries and irrigation channels. The fish attains a fairly large size and is of a great commercial value and some authors have ranked this face as only next to 'hilisa' in the Ganga river system (Saigal and Motwani, 1961) [11]. Most of the studies have evaluated the biochemical composition and nutritive value of muscles however there is scarcity of data in other tissue such as liver and ovary and their seasonal variation (Bhagowati AK, 1982) [4]. The present study deals with the changes in the biochemical constituents of its muscles, liver and ovary from season to season.
The feeding intensity of the fish varies with the season and is related with maturity, spawning and the availability of food items. Several investigators have found that the feeding intensity of the mature fish decreased during the spawning season as compared to the other month of the year. The feeding intensity increases after spawning but during the spawning period stomach is either empty or only ¼ th full, which may be due to considerable increase in the size of the gonads. Feeding intensity has also been reported to be correlated with the availability of the food items in the environment (Singh and Bahuguna, 1983) [12]. Gastrosomatic index of several species shows seasonal variations in both the sexes and is maximum during post spawning period and minimum during breeding season (Ashraf Abbas. 2010) [3].
Changes in the body composition and protein mass of fishes have been associated with seasons and demonstrated in some species.
However, few investigators have studied the seasonal biochemical changes in the ovaries and other tissues of fresh water fishes. Seasonal changes in the ovary and ovarian cycle have been described by Khanna and Pant (1967) [8, 11]. in *Glyptosternumpectinopternum* however seasonal and developmental biochemical changes in certain tissues and ovarian cycle in fishes inhabiting the running water of Vindhya region have not been described. Keeping in view the economic importance of *Aorichthys seenghala*, the present study was undertaken to evaluate seasonal variation in biochemical composition of different tissues. The results would form a basis in taking up positive steps for management and conservation of this important food fish in Vindhya region.
Various literature have shown that the food and feeding habit of fish has its impact on the various biochemical components of the fish such as protein, fat, carbohydrate, moisture, ash and iron contents in the liver muscles and the ovary of fish (Craig FJ, 1978) [5]. The glycogen forms an energy source during ovarian cycle and must have a definite relationship in the above state parameters and spectacular variations may occur during the

different months of the year in accordance with the feeding habit and ovarian cycle. Determination of biochemical composition will further help to optimize the proper growth of fish and manage fish culture. Not much work has been done on the biochemical composition of the flesh or the organs of the *M. seenghala*. The work reported here was undertaken to study the correlation of food and feeding habits with the seasonal variation in the fat, carbohydrates, proteins, moisture, and ash and Iron content in the liver, muscle and ovary during entire ovarian cycle in an economically important fresh water fish *Mystus seenghala* (sykes).

2. Material and methods

Collection of Fish

Living specimens of *Mystus seenghala* was collected from Tons river and other fresh water resources of Rewa Region (M.P., India). The specimens were also procured from local fish market during different months of the year for studying the food and feeding habits of this species.

Food and feeding habits

The specimens collected from July to September 2004 were utilized for preliminary studies. The specimens collected from October 2004 to January 2006 were analyzed quantitatively for elucidating seasonal variations in the food components. These data were also analyzed for various arbitrary size groups in order to see if there is any basic change in dietary habits of the fish at various stages of its growth. Since the number of specimens in similar size groups was considered inadequate, the guts from 312 additional specimens, measuring 18 to 215 mm, were examined from October 2004 to January 2006. However, the observations on the composition of gut contents of various size groups were limited to the period from October to January as all size groups were adequately represented during this period only. The guts were removed from fresh specimens of *Mystus seenghala* and preserved in 5% formalin for subsequent analysis. The contents of the preserved guts were teased in petridishes in order to render their microscopic examination easy. Since the most portion of the alimentary canal of this species is not differentiated into a recognizable stomach, the contents, sampled at random, were examined from various sections of the guts. The gut contents were analyzed by volumetric method, as well as by frequency of their occurrence in the guts. The volume of the gut contents was estimated by a modified point's method, which is described in the following section. In the occurrence method, the number of specimens in which a particular item occurred was given as a percentage of the total number of specimens examined. The occurrence percentage of a particular item was also calculated with reference to the sum of occurrences of all the items (Adeyemi SO, 2009) [2].

New technique for volumetric analysis

The guts of 86 specimens of *M. seenghala* collected from October 2004 to January 2006 were examined, with a view to get familiarized with food organisms encountered in its guts and to evolve a suitable and dependable technique for assessing its food components volumetrically.

The volumetric assessment of planktonic organisms is generally done either by eye estimation or by point's method, in which the bulk of various food organisms is arbitrarily determined. In other methods the organisms are either merely listed or counted. On the basis of preliminary observations on the gut contents of *M. seenghala* a new technique for volumetric food analysis, which is essentially a 'points method' (Swynnerton and Worthington, 1940) [13], was devised for assessing the food composition of this species.

In the point's methods, adopted by Swynnerton and Worthington (1940) [13] and quoted by Hynes (1950) [6], "the food items in each fish stomach were listed as common, frequent, etc. on the basis of rough counts and judgment by eye, due regard being taken of the size of the organisms as well as of their abundance. Each category was the allotted a number of points and all the points gained by each food item were summed and scaled down to percentages to give percentage composition of the food of all the fish examined." One of the criticisms of the points methods, as pointed out by Hynes (1950) [6], is that 'it is subjective and the investigator may be influenced by prejudice in his allotment of points'. This criticism was largely overcome in the present study by suitably modifying the 'point's method' to render the volumetric food analysis objective. The modified method is described below.

The outlines of all the teleosts, Insects and Crustaceans organisms observed in the guts of *Mystus seenghala* were drawn on a graph paper of same scale with the help of camera lucida and by using the same lens combination of a particular microscope. Complete squares enclosed by the outline of each organism were counted. The marginal squares of the outlines which enclosed more than half area were counted and those with less than half area were ignored. Among the food organisms, *Navicula*, a diatom, was found to be smallest and so taken as a unit and assigned one point. The variations in size and thickness of an organism were assumed to be negligible and therefore were not considered for the volume estimations by this method. In the case of Crustaceans such as *Elmis*sp., *Coleoptera larvae*, *Chiromonas*sp. And *Odontata nymph* where the total lengths varied considerably, the number of squares enclosed by a single cell were counted and points accordingly assigned. The points once assigned to various organisms were used throughout the investigation. In the actual analysis by this method the various organism in a sample were counted separately and the total number of individual organisms was then multiplied with their respective points to arrive at the total of all points gained by them. The gut contents of *M. seenghala* broadly consisted of Teleosts, Insects Crustaceans and mud mixed with sand. The percentages of these broad groups were first assigned by 'eye estimation' as no other method was feasible for the volumetric analysis of all these groups. The percentages of various Teleosts as determined on the basis of points gained by them were then scaled down making up the total percentage of the Teleosts group previously assigned by eye estimation. The technique, as described above, is most suited for the volumetric assessment of the food components of fishes like *M. seenghala* which subsist mainly on Teleosts as well as for volumetric analysis of Teleosts in hydrological studies.

Determination of biochemical Component

For the biochemical study, attempt has been made to estimate the changes in the organic and inorganic component such as quantity of protein, lipids and carbohydrates, moisture, ash and iron in the muscle, liver and ovaries of *Mystus seenghala* during different seasons. Immediately after collection of fishes were sacrificed after pitching. The ovaries, muscle and liver were taken out and were put into the crushed ice and weighed to the

nearest gram. The following methods were employed for different estimation.

Estimation of Protein

Protein was estimated by biuret method as cited in Hawk's physiological chemistry (edited by Oser, B.L., 1965) [5]. In brief 0.4, 0.8, 1.2, 1.6 and 2.0 ml. of standard protein solution was kept in a series of tubes. The volume of each tube was made up to 2.0 ml. with water, and then to it, was added 8.0 ml. of Biuret reagent. It was then thoroughly mixed. After 30 minutes, optical density was recorded against blank at 670nm.

Determination of Lipids

Lipids were extracted with chloroform using soxhlet apparatus. Method of Weil and Stetten (1947) [14]. 100 mg. of the tissue was introduced into the fractioning column of the soxhlet extractor. The flask containing chloroform was slowly heated in a water bath until the chloroform in the fractioning column become colorless. The apparatus was disconnected and the tissue was accurately weighed after evaporating the chloroform in an oven at 47ºC. The difference in the weight of tissue before and after evaporation with chloroform gave the lipid content of the tissue in milligrams (Ackman RG, 1990) [1].

Determination of Glucose

For the estimation of glucose, Nelson (1944) [9] and Somogyi (1945) [9] method was used. In brief 50 mg of tissue was taken in a Folin-Wu-tube and to it was added two ml. of Somogyi's copper reagent. Tube was placed in boiling water for 20 minutes. Tube was allowed to cool at room temperature and then 2 ml. of Nelson's arsenomolybdate solution was added. The solutions were effectively mixed by moderate vertical agitation with a small knob on the end of a glass rod. The contents of the tube were diluted to the 25 ml. mark with water before shaking to ensure through mixing. The tubes were then allowed to stand for 15 minutes to permit maximum color development. The solution was read at 660 nm in a photoelectric colorimeter. With each series of unknown samples, tubes containing 2 ml of water of for a blank were treated and 2 ml of standard solution having 0.10 and 0.20 mg of glucose in a similar manner to obtain a standard reference graph.

Determination of Iron

Method of Kennedy (1927) was used for the biochemical concentration of iron. In brief 50 mg. of tissue was taken in a hard glass tube and 5.0 ml. of concentrated sulphuric acid and 1.0 ml. of 60% perchloric acid were added to it. The contents were heated for about 10 minutes till the tissue become black. The tubes were cooled and a drop of concentrated nitric acid was added to complete the digestion. The solution thus obtained was diluted to 10.0 ml. with 10% sulphuric acid to 1.0 ml. of this solution corresponding 5 mg. of the tissue and 1.0 ml of standards containing 1 mg. and 2 mg respectively, 9.0 ml of 10% sulphuric acid. 5.0 ml. of 20% potassium thiocyanate and 10.0 ml. of isoamyl alcohol were added and the solution was thoroughly mixed. The coloured alcoholic layer was separated and measured in EEL photoelectric colori-meter at 540. The instrument was set to zero with 10 ml. of isoamyl alcohol. A calibration curve was plotted from the optical density of the two standards, by which the final amount of iron in unknown solution was calculated.

Determination of Moisture and Ash

For the determination of moisture and ash percentage, the methods were followed as recommended by the Analytical Method Committee (1979).

Moisture

A portion of the prepared samples were mixed (in separate silica dishes) with sand and ethanol, the ethanol is removed by gentle heating on a water bath and the mixture was then dried to constant mass to 103 $\pm$ 2ºC for 2 hrs.

Calculations

$$\text{Moisture Content (\%)} = \frac{m_1 - m_2}{m_1 - m_0} \times 100$$

Where,

m0 g = mass of dish, rod, sand
m1 g = mass of dish, rod, sand and sample before drying
m2 g = mass of dish, rod, sand and sample after drying

Ash

Prepared sample in a pre-weighted dish was heated with 1 ml 25% m/v magnesium acetate solution on water bath, when dry, incinerated the mass at 550–600ºC for at least 3 hrs. (To white ash), and allowed it to cool in a desiccator and weighed the ash by a named electronic balance.

Calculations

$$\text{Ash content (\%)} = \frac{m_3 - m_1 - m_4}{m_2 - m_1} \times 100$$

Where,
m_1g = mass of dish
m_2 g = mass of dish and sample
m_3 g = mass of dish and residue after drying
m_4 g = mass of magnesium oxide originating from the Volume of magnesium acetate solution added.

3. Results and Discussion:

3.1 Evaluation of Food and Feeding Habits

State of feed: The condition of feed of 343 adults (Fig. 1) fails to indicate any marked season of intensive feeding. Presuming fish with "Gorged", full; ¾ full' and '1/2 full 'condition of stomach to have fed actively, it is seen that during July to September and December the fish is feeding actively as compared to the rest of the period. The percentage of empty stomachs is appreciably high throughout the year. Mature specimens examined during breeding season had almost empty guts.

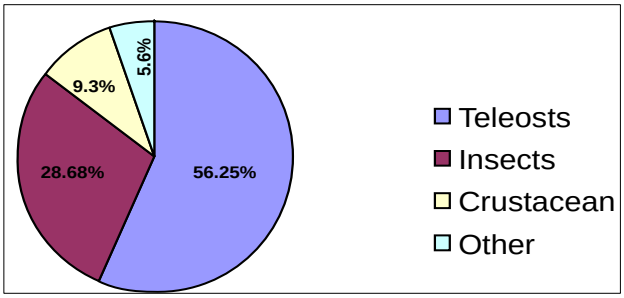

Fig1. Diagram showing the volumetric composition of food in the gut of adult female *M. seenghalal.*

Analysis of Gut content

Gut content was analyzed by determining the different food such as teleost, crustacean and insects. Figure 1 shows that 56.25 % of the total volume of food of fish consisted of teleosts and was consumed by 56.15% of the fish examined. The insect formed 28.68% of the total Gut contents and 38.27% of the fish examined had subsisted on them. The crustaceans, next in order of abundance formed 9.31% items of food were plant matter and Detritus forming 5.76% of the total food and 19.70 % of the specimens had consumed them.

Teleosts

The teleosts could be readily identified by the skeletal remains. There specific identification often became difficult due to their being in advanced stages of digestion in stomach. A few cases of cannibalism were observed and the smallest and cannibalistic adult, recorded in the month of July was 536mmin total length having two specimens of 132 and 143 mm length of *M. seenghala* in the gut. Incidentally this was the largest size of fish eaten. The monthly fluctuation in the quantities of teleosts as percentage of total volume of feed and the forms that constituted them are presented in the tab 1. Teleost forms the bulk of food during May to November and relatively low quantities of teleost were consumed during January to March and December. The frequency of occurrence of teleosts was higher in the month of June to October and lowest in the month of February and March.

Insects

These could be identified from general exoskeletal characteristic. Larvae and nymphs were identified by the characteristic features of different groups. Only generic identification was possible. The insect mainly consisted of the larvae and the nymphs of odanata, Diptera, Mecoptera, Tricoptera and celeptera. In few cases adult aquatic insect were also encountered. Monthly variation in the quantity of insect and their forms appear in tab.1 except during July to September insect are consumed by the fish throughout the year. During April, May and November their quantities were extremely low. Complete absence of insect in July to September is probably due to heavy inflow of Freshets and resulting high turbidity. The degree of prevalence of insect is higher in March and lowest in October.

Crustaceans

The crustaceans comprising of prawns and shrimps (palaemonlamarriileanderstyliferus) and crabs (varuna sp.) formed 9.31% of total feed. Considerable quantities of crustaceans were consumed in February, October and November and they were completely absent in June, September and December. The frequency of prevalence was higher during July, August, October and November (Fig. 2).

Plant matter and detritus

Bits of leaves and tender shoots of aquatic plants, dried twigs and leaves of other plants that are occasionally washed off in the river waters and the decaying organic matter constituted the plant matter detritus. Only 19.5% of the guts examined contained it and formed 5.76 % of the total feed. In January, March, November and December sufficient quantities of plant matter and detritus was eaten (fig3). The low percentage by volume and comparative higher percentage by occurrence throughout the year except for July and September when plant matter and detritus is not found in the guts, suggests that these are only accidentally taken by the fish while feeding on other major food items. Besides the above food items, the presence of sand grains, mud and small pebbles even in larger quantities is not an uncommon feature. Sand and mud is accidentally taken in while on other food items particularly the insects and crustaceans. The presence of sand and mud in the stomach also indicates the bottom feeding habits of the fish.

Table 1: The state of feed (%) of M (O) examined from Ganga River System at Allahabad.

Month	Gorged	½Full	½Full	½Full	½Full	With title food	Very little food	Empty
January	..	..	4.0	4.0	24.0	48.0	4.0	16.0
Februay	..	6.2	…	25.0	37.5	12.5	..	18.8
March	7.7	15.4	..	..	30.7	23.1	..	23.1
April	..	..	3.6	3.6	3.6	42.8	10.7	35.7
May	3.0	3.0	..	9.1	3.0	36.4	12.1	33.4
June	7.1	..	3.2	25.0	25.0	21.4	7.1	10.2
July	4.8	14.3	4.8	19.1	14.3	..	9.55	33.2
August	..	10.0	20.0	30.0	10.0	10.0	10.0	10.0
September	..	..	11.2	33.2	22.2	11.2	..	22.2
October	..	..	13.4	13.4	26.6	20.0	6.6	20.0
November	4.2	4.2	4.2	12.5	8.3	12.5	16.6	37.5
December	9.5	4.8	9.5	19.3	14.3	14.3	..	28.6

3.2 Determination of seasonal fluctuation of carbohydrate content

Carbohydrate content was determined in ovary, liver and muscles. Table 2 showed the changes in the carbohydrate content of different tissues.

Ovary

Highest level of glucose was recorded in 6.55 ±0.04 mg/g in ovary in the month of July, which gradually reduces, and lowest recorded in January (2.32 ±0.05 mg/g). Considering these data it appears that carbohydrate content of ovary is perhaps

synthesized from the monomer depleted from the ovary does not include any visible carbohydrate content during the resting phase. This is due to probably the exogenous synthesis of carbohydrates yolk of *Aorichthys seenghala* in mature oocytes, which are abundantly present in the spawning period.

Liver

In liver the glucose content was found to vary considerably from season to season. Highest level of glucose (30.16± 1mg/g) was recorded in the month of January, which gradually reduces, and lowest recorded in June (4.85 ±.05 mg/g) and then again increases to (27.88 ±.08 mg/g) in the month of December.

Muscles

Muscular tissue constitutes about 40% of the body weight and it is therefore the largest single tissue component of the body, which can suddenly change its metabolism manifold depending on its state of activity. The activity of muscles is major determinant of metabolic requirements of the body and all the circulatory and other adjustment related to this. The occurrence of carbohydrates in the muscles is not constant. The highest amount of glucose in the in the ovary (6.55 mg/g) and its lowest level in liver (0.85 mg/g) can be rerecorded during the spawning phase. The lowest glucose level in the ovary (2.32 mg/g) and its highest level in the liver (30.62 mg/g) are noted in the resting /spent phase. Glycogen quantity changes with maturation cycle of the ovary. The lowest quantity of glycogen was observed in stage II and in the following state. Glycogen content reaches to its maximum in stage IV.

Table 2: Seasonal fluctuation of the Carbohydrates content in the liver, Muscles and Ovary of *M. seenghala*.

Months	Liver	Muscles	Ovary
January	46.15±3.89	10.25±0.2	4.26±0.35
February	38.34±3.45	15.32±1.0	4.88±0.32
March	33.26±2.69	8.02±1.04	7.10±0.23
April	29.24±3.0	9.02±0.59	10.85±0.88
May	25.38±4.25	10.54±0.25	13.17±1.0
June	24.58±3.54	13.2±0.65	15.68±1.87
July	23.89±2.89	12.23±1.69	15.02±2.33
August	24.25±2.48	10.36±2.0	13.86±2.80
September	30.02±5.0	11.25±0.89	5.10±0.97
October	29.03±5.03	14.04±0.	234.39±0.77
November	40.16±6.06	15.3±2.14	4.10±0.45
December	41.25±5.07	15.2±1.56	4.22±0.12

3.3 Determination of seasonal fluctuation of fat content

Fat content was determined in ovary, liver and muscles. Table 3 shows the changes in the fat content of different tissues.

Muscle

Fat cycle in muscle was not very well defined. It recorded maximum values during October where as in November there was a distinct fall which was followed by a rise during January and February. Relatively low values were recorded in subsequent months (March to August).

Liver

The fat content in liver was higher than that of the muscle and was subjected to significant variations from season to season. These has been given in the Table 3 and plotted in fig.-8. There were two phases of maximum and minimum values of liver fat. Higher values were recorded from about August to November. This was followed by relatively low values during the winter months (December to January). The next phase of maximum fat was in April and minimum in May. The curves for the male and female were almost identical and there was no quantitative difference between the liver fat of the two sexes.

Table 3: Seasonal fluctuation of the Fat content in the liver, Muscles and Ovary of *M. seenghala*.

Months	Liver	Muscles	Ovary
January	2.892±0.09	0.645±0.01	0.785±0.06
February	3.036±0.89	0.669±0.02	0.706±0.35
March	3.975±0.75	0.301±0.03	0.892±0.25
April	4.578±0.65	0.331±0.05	1.135±0.35
May	3.025±0.69	0.271±0.08	1.167±0.24
June	3.967±0.58	0.41±0.05	1.343±0.25
July	4.563±0.78	0.19±0.06	0.872±0.24
August	4.835±0.38	0.36±0.02	0.423±0.31
September	5.047±0.65	0.6±0.01	0.457±0.32
October	4.976±0.54	1.21±0.04	0.738±0.39
November	4.0265±0.25	0.3±0.06	0.985±0.29
December	2.961±0.28	0.295±0.09	0.692±0.02

The fat cycle of the liver does not indicate much relationship with that of the muscle except that high aft values occur in both the tissues during October and February.

Ovary

The changes in the ovarian fat were more marked than that of the muscle or liver (Tab. 3). Fat values were low during November and December but after that there was a gradual rise. Highest values were noted in June and the lowest values in August for the ovaries of *M. seenghala* contents less fat than the testes. This is probably because in the ovaries of cat fishes it is the protein (nucleoproteins) rather than the fat reserves which remains more dominant. A comparison of the ovarian fat cycles with the fat cycle of muscle will indicate that high ovarian fat cycle from March to onwards coincide with the low muscle fat values. Similarly low ovarian fat value during the post monsoon and winter months corresponds with the high muscle fat values. In liver peek fat values proceeds the peek obtained for the ovaries.

A gradual storage of the so-called visceral in the body of *M. seenghala* was noted throughout the present study. This was gradually depleted during the summer months. Deposition of adipose layer was also noticed below the skin especially caudal region. No quantitative estimation could be made for these extra fat stores but from a gradual concluded that these fat reserves contributes substantially towards the energy demands of the developing ovary.

3.4 Determination of seasonal fluctuation of protein content

Protein content was determined in ovary, liver and muscles. Changes in the protein content of different tissues were as follows

Muscles

Seasonal changes in the muscles protein have been given in (table 4). As observed from the table variation in the muscles were not pronounced. They followed a well-marked seasonal cycle uniformly low values were recorded during the months

(November to February). A rapid building up of protein was noticed from March onwards and the highest were attended in July. This was followed by a gradual fall in the subsequent months. A comparison of the muscles protein cycle with the fat cycle described earlier would reveals that the two cycles were more or less inversely related.

Table 4: Seasonal fluctuation of the Protein content in the liver, Muscles and Ovary of *M. seenghala*.

Months	Liver	Muscles	Ovary
January	9.073±0.98	13.618±0.98	7.678±1.30
February	10.231±0.88	13.012±0.84	9.895±2.65
March	12.432±0.89	16.056±0.76	11.215±3.45
April	15.393±0.97	16.532±0.8	13.214±4.0
May	15.086±1.65	18.609±0.89	21.816±4.2
June	15.407±2.03	19.239±0.99	21.972±6.0
July	15.237±2.90	17.617±1.02	13.278±5.23
August	15.832±3.45	17.327±1.35	11.325±2.80
September	15.153±3.25	17.048±2.0	12.462±3.60
October	11.812±1.98	15.065±2.30	10.931±4.0
November	13.372±4.02	13.956±3.01	11.132±2.35
December	10.464±1.50	13.783±1.99	9.689±3.6

High muscles protein values from March onwards coincides with the low values of fat and vice versa. In *M. seenghala* the protein values remain uniformly low throughout the winter months.

Ovary

Monthly ovarian protein values have been given table 4. The values of ovarian protein were low from November to January and high during May to July. A rapid fall was recorded from august which reached its minimum in October. The rise and fall in ovarian protein and ovarian fat were alike the cycle of protein in ovary and muscles were almost similar but in the liver a uniformly high protein was maintained for a longer period.

Liver

A well-defined seasonal cycle was observed in the liver protein (table 4). In *M. seenghala* the protein values were lowest in January but gradually increases from February onwards reaching about the maximum in April. The values remain steady until September.

A comparison of the protein cycle in liver and muscle would indicate that in both the tissues the values remained high during summer and monsoon months (April to September) maximum values were attained earlier in the liver and continued to remain high for several months afterwards. In the muscles, on the contrary the values fell immediately after reaching peak in June.

3.5 Determination of seasonal fluctuation of moisture content:

Moister content was determined in ovary, liver and muscles. Changes in the moister content of different tissues were as follows

Muscle

Moisture cycle in muscle was less defined (table 5).Low moisture values were noted during January and June and in October the values were lowest. The fall in moisture during these months was associated with a rise in the fat values. The highest moisture percentages were noted during July.

Liver

Monthly values of the moisture in the liver are given in the table 5. The values were generally lower than those of the muscle. There were two phases of maximum and minimum moisture values which were found to alternate inversely with the liver fat. High values were noted from December to January and again from May to July. Very low values were recorded in April again from August to November.

Table 5: Seasonal fluctuation of the Moisture content in the liver, Muscles and Ovary of *M. seenghala*.

Months	Liver	Muscles	Ovary
January	78.763±0.98	78.327±0.8	82.97±0.89
February	77.036±0.58	78.136±0.9	82.652±0.98
March	77.65±0.69	79.612±0.56	80.766±0.72
April	75.968±0.78	79.309±0.45	75.327±0.39
May	77.723±0.36	78.084±0.36	74.037±1.04
June	77.215±0.65	79.998±0.4	74.192±1.15
July	78.678±0.64	79.145±0.58	80.275±0.85
August	75.895±0.25	78.153±0.6	83.087±0.73
September	75.146±0.29	77.872±0.58	82.034±0.65
October	76.21±0.58	78.975±0.3	81.357±0.72
November	74.173±0.47	79.953±0.36	82.165±1.3
December	77.894±0.69	79.226±0.45	83.215±1.25

Ovary

Table 5 gives the monthly values of moisture in ovary. It can be seen from the figure that the moisture cycles were fairly well marked in ovaries and showed a reciprocal relationship with the fat cycle. The values were fairly high during winter months (November to February) but declined rapidly in the following month, the lowest being in May and June. A sudden increase in moisture was again noticed in July and the values remained high in subsequent months.

3.6 Determination of seasonal fluctuation of ash content

Ash content was determined in ovary, liver and muscles. Changes in the ash content of different tissues were as follows

Muscle

Seasonal variations in the ash content of muscle were not very well marked (table 6).The range was from 1.0 to 1.7% low as values were recorded during the winter months (November to February) and high values in August.

Table 6: Monthly fluctuation of Ash content in Liver, Muscles and Ovary of *M. seenghala* as per 100mg of fresh tissues.

Months	Liver	Muscles	Ovary
January	1.407±0.089	1.023±0.055	1.636±0.24
February	1.442±0.024	1.078±0.041	1.847±0.54
March	1.560±0.35	1.162±0.032	1.628±0.54
April	1.548±0.256	1.213±0.021	1.731±0.91
May	1.361±0.254	1.223±0.012	1.508±0.78
June	1.928±0.0365	1.425±0.11	1.667±0.35
July	1.392±0.025	1.359±0.015	2.246±0.78
August	1.490±0.045	1.597±0.045	2.197±0.41
September	1.572±0.0251	1.427±0.029	2.253±0.88
October	1.583±0.056	1.281±0.034	2.021±0.87
November	1.636±0.088	1.132±0.28	1.652±0.73
December	1.521±0.057	1.179±0.56	1.032±0.049

Liver

Little variations in the ash content of liver were noticed (Tab. 6). There were two periods of alternating maximum and minimum

values. High values were obtained from August to November and from March to April and low values from December to January and May to July.

Ovary

Ash content could be estimated in ovaries and these are given in table 6and fig 20. The ash values showed considerable fluctuations from month to month. Highest values were recorded from July to September and lowest in May. The ovaries contained higher ash values than the liver or the muscle.

3.7 Determination of seasonal fluctuation of iron content

Fat content was determined in ovary, liver and muscles. Changes in the iron content of different tissues were as follows

Muscle

High iron content was recorded in the muscle of *M. seenghala* but there was not much variation from month to month (Tab. 7) slightly high values were noted from September to December.

Table 7: Monthly fluctuation of Iron content in liver and Muscles of *M. seenghala* as per 100mg of fresh tissues.

Months	Liver	Muscles
January	40.03±4.89	19.12±2.14
February	51.79±7.12	22.87±1.24
March	43.25±4.36	19.13±1.34
April	20.85±2.54	18.89±0.98
May	20.61±1.87	17.12±1.65
June	39.03±4.01	25.37±2.15
July	55.07±4.36	24.08±0.87
August	57.32±4.56	30.98±1.5
September	68.45±4.69	24.14±2.0
October	44.17±2.6	25.30±1.78
November	43.78±2.41	24.14±1.54
December	25.82±1.99	20.670±1.45

Liver

Liver showed even more iron content than the muscle (Tab. 7) but here also no distinct seasonal cycle could be established. Higher values were, however recorded from July to September and low values during April and MayVarious studies have shown that the iron content is related to the sexual maturity of the fish (Kailasam t al., 2015)

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