Seasonal Changes in GSI, Fecundity and Biochemical Composition of *Clarias batrachus* (Linnaeus).

A Thesis

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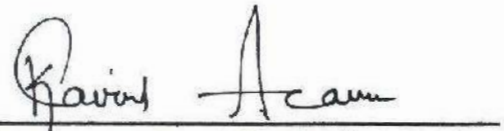
Approved as to style and content by



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DECLARATION

The results submitted in this thesis are entirely of the candidate's own investigation and no part of the results has been accepted for any degree, nor it is being concurrently submitted for any degree.

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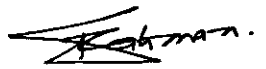

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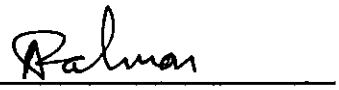

Mohammad Abdur Rahman

DEDICATED TO

My Beloved Parents

Acknowledgement

All praises are to the supreme being, creator and ruler of the universe, whose mercy keep me alive and enable me to pursue are education in Fisheries and Marine Resource Technology Discipline (FMRT) and to complete the thesis for the fulfillment of the degree of Masters of Science in Fisheries and Marine Resource Technology.

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Abstract

To classify the biology of the *Clarias batrachus*, in Khulna region, sex ratio, monthly changes in GSI and fecundity were observed in the Laboratory. Male-female ratio of the studied species was 1: 0.94. Fecundity of the fish ranged from 1875 to 10798 in the total length ranged of 22.3-23.7 cm. The fecundity is more directly related to the ovary weight. The regression line of fecundity in total length, standard length and gonad weight were all found to be linear and their co-efficient were all positive and significant at 5% level of significance. But the regression line of fecundity in body weight was negative. From their monthly dynamics in GSI, spawning seemed to have taken place somewhere between May to July with a peak in June for male and between June to August with a peak in June for female. Furthermore, GSI revealed that *Clarias batrachus* has a short spawning season.

For different body parts, the protein content was higher in the middle portion of male (19.17 ± 0.303%) and in female it was (19.03 ± 0.131) in tail; the lipid content was higher in middle portion of male (1.19 ± 0.270) and female (1.10 ± 0.213). The percentage of ash content was highest in the head portion of male (12.88 ± 0.177) and female (12.20 ± 0.046), moisture content was highest in middle (79.31 ± 0.238%) and lowest in head (69.87 ± 0.270%) of male. For female the highest moisture content was in the middle (80.43 ± 0.15) and lowest was in the head (71.9 ± 0.136).

Annual changes in nutrient content like protein, lipid, ash and moisture were assessed in the *Clarias batrachus* in both wet weight (body flesh) and dry weight (whole body) basis during the month May 2002 to April 2003. The highest value of protein in the body flesh was found in October (21.75 ± 3.897%) for Male and in August (20.303 ± 0.040%) for female. The highest value of lipid was found in December for male (5.038 ± 1.314%) and for female the value was in the month of January (5.672 ± 0.064%). The highest value of ash was found in October (2.053 ± 0.632%) for male and September (1.825 ± 0.154%) for female. The highest value of moisture content was found in July for male (80.01 ± 0.085%) and for female the in was in the month of April (80.33 ± 0.129%). For whole body, the highest value of protein (19.322 ± 0.901%), lipid (7.761 ± 0.66%), ash (5.613 ± 0.532%) and moisture (79.13 ±

0.163%) was found in December, October, December and July, respectively for male. On the other hand, the highest value of protein (18.899 ± 0.154%), lipid (5.10 ± 0.028%), ash (5.430 ± 0.037%) and moisture (76.72 ± 0.276%) was found in July, May, June and October, respectively for female fish. From the nutritional point of view it shows that the nutrient content of *Clarias batrachus* is undeniable compared to other small and large fishes.

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Chapter 1

General Introduction

1. Introduction:

Bangladesh has a geographical area of 1.4 million Km^2 with an estimated population of about 120 million. Her vast water area includes 43.1 million ha of fresh water which is an excellent ecological condition for the propagation of fish and related organism (BBS 1994). Fish is one of the most important export items of the country that contribute about 6% of the export earning and 5.3% to GDP. About 63% animal protein is supplied from fish (DOF-2002). The importance of fish for consumption is increasing rapidly because of increased population. As a result daily per capita consumption of fish has drooped 26 gm/day, which is required 38 gm/day. Per capita fish consumption is needed 18 kg/annum, where as the actual picture is 12 kg/annum (DOF-2002). About 80% of our annual catch comes from inland waters of which are breathing fish like singi, magur, koi account for about 20% of the total catch in Bangladesh (GOB 1984).

Bangladesh steps in a new millennium with high hopes and aspirations, strengths and weakness and new opportunities and constraints for its development. Fisheries sector plays a vital role from the point of nutrition supply, employment generations, foreign exchange earning and other areas of economy. The total production from the freshwater resources of our country is very high in the world except from China and India, producing more inland fish than Bangladesh (Nuruzzaman, 1987).

The country is extremely flat and intercepted by thousand of braches of three main rivers. Every year when snow on the Himalayas melt, the onrush of water passes through the country gradually overflowing the bank of these rivers and flooding a large portion of the country. The flooded lands are extremely fertile because of deposited sediment and provide a rich habitat for freshwater tropical fish. Beels are excellent natural habitat for large and small indigenous fishes of different food habit (Mustafa *et al.*, 1983).

Among the aquatic lives, the number of freshwater indigenous fish species has been recorded a total of 260 including some brackish water species (Rahman, 1989). Recently, FAP17 (1994) identified 231 species in Bangladesh from several freshwater habitats. Most of these are small and were abundant in all the compartment of the aquatic habitat.

Prior to 1970, many different small indigenous fishes like Koi, Taki, Vada, Pabda, Gulsa, Mola, Puti, Shing, Magur etc. were abundant in almost all the freshwater areas of Bangladesh. They were commonly caught by the large number of subsistence fishermen and provided a major portion of their protein intake. Moreover, these indigenous fish were favorite and popular for their taste (Moniruzzaman, 2000).

Live fishes like *Heteropneustes fossilis, Clarias batrachus, Anabas testudeneus* etc, accounted for about 20 percent of the total catch in Bangladesh. (Ali *et al,* 1991) Catfish *Clarias batrachus* (Linn.) locally know as magur belongs to the family Clariidae. It is one of the most popular food fish among all fish species and widely distributed all of Bangladesh in rivers, haor, baors, beels, jheels, canals and ponds. Once easily available in nature the fish has, recently became scarce because of many adverse changes in their natural breeding and growing habitats. For this reason, *Carious batrachus* fry in very rare in nature. Until recently, the supply of its fry comes from natural sources. This is one of the living factors towards catfish farming (Master *et. al.*, 1954).

Small fishes and catfishes are paying a vital role in the inland fisheries of Bangladesh. This vital sector seems to have received little attention and public policy support. Most of the catfishes (*Clarias batrachus, Heteropneustes fossilis, Mystus vittatus*) can withstand harsh environmental condition (Nuruzzaman, 1987) such as, low oxygen level, wide fluctuation in temperature and other adverse conditions. Above all, they enjoy wide acceptance as food fish due to their portability and marketability.

Clarias batrachus is usually confined to stagnant, muddy water are generally found in association with members of another closely related species, *Heteropneustes fossilis* the air-breathing organs occupying the upper part of the branchial cavity not only enable them to live in very oxygen poor water but also permit them to exist for hours at a time out of water. It breeds during May through July with the approach of monsoon in shallow waters in inundated paddy fields and similar body of water (Rahman, 1989).

Intensive study of various aspect of biology of fish such as sex ratio, fecundity, gonado-somatic index etc is of crucial important in fishery management. Analysis of proximate composition including moisture, protein, fat, and ash is very important to evaluate food

value and also to find out seasonal quantitative variation in order to correlate with the gonadal variation. Proximate analysis is an index of nutritive value only because the fractions it isolates are correlated with some of the properties of organisms that are nutritionally significant.

Clarias batrachus occupies the best position of all the fresh water catfishes. Although this fish is commercially important but few research have so far been done on the sex ratio, gonado-somatic index, fecundity and proximate composition. Hence this species has been chosen for investigation of its biology and proximate composition for the following purposes.

- To know details information about sex ratio, gonado-somatic index and fecundity.
- To assess the annual changes of proximate composition.

Chapter 2

Biology

2.1 Biology:

Introduction:

Bangladesh is blessed with vast natural resources which is full of fishery resources. But the fishery resources are depleted every day due to water pollution, physiochemical change of water and above all improper fisheries management and conservation measure. Bangladesh is one of the populous countries in the world. Her population is increasing day by day at an alarming rate in comparison with land. As the days proceed the nutritious foods are decreasing with the increase of population of Bangladesh. Above 75% people of Bangladesh have been suffering from malnutrition (DoF, 2001). So in the new millennium, the greatest challenge of Bangladesh is to combat the prevalent malnutrition and it is possible to fight against such sort of challenge by proper utilization of biological resources, especially fisheries resources.

Fish is an excellent source of protein. It was a vital item in the diet of poor people of our country. Fish had been in plentiful supply and a fish based diet was part and parcel of Bangali dietary culture. There is a proverb that "macha-vate Bangali". But over the last several decades fish was becoming scarcer day by day.

Study of fish biology such as sex ratio fecundity, gonado-somatic index etc. is very essential to get the information about maturation, spawning period, breeding (artificial and natural) and availability. Fishermen have no knowledge about breeding season of the particular species and they engage in fishing even in breeding period. Therefore, there is a need, not only to culture of the species but also conserve the fisheries resources of the country.

Clarias batrachus is an indigenous species. Once it was available in nature. But now its availability is decreasing due to habitat destruction and other causes. But this fish offers high nutritional value and it is very much tasty (Rahman, 1989). Hence the importance of its culture is needless to say.

Asian catfish, *Clarias batrachus* preferred choice of fish among Bangladeshi people because of its taste, no contra muscular bones and high nutritive value. Consequently, people willingly pay high price. Recent estimates suggest that worldwide 20% of all fresh

water species are extinct, endangered or vulnerable. As result, fish stock particularly those dwelling in inland open waters, have gradually become endangered. IUCN, Bangladesh (1998) has documented about 56 fresh water fish species critically or somewhat endangered. Cat fish is no exceptional. Yet there has been little research on various biological aspects of this species. Considering the importance and demand of the people of the country, investigation on some aspects of biology, like, sex ratio, fecundity and GSI of *Clarias batrachus* has been undertaken to create awareness among the consumers and policymakers of the government. The overall objectives of the study were as follows.

- To determine the sex ratio of the species in natural population.
- To know the exact number of fecundity present in the ovaries which is highly important to evaluate the recruitment pattern.
- To observe the maturity and exact time (season) of spawning which is essential for providing better understanding in spawning.

2.2 Review of literature:

Prior to conduct an experiment, it is essential to know the information about the previous related works. So far as, Bangladesh is concerned there is a little publication on the different aspects of biology of *Clarias batrachus*. The following information was reviewed in favor of the present study, which was performed elsewhere in the world and relevant to the study.

A number of authors established some relationship between fecundity and total length, body weight and gonad weight. Bagenal (1966) reported that the relationship between fecundity and length for most species was curvilinear. Pillay (1958) found the linear relationship between fecundity and the body weight of hilsa, and the relationship between fecundity and length was curvilinear. However, there are also reports of having a linear relationship between the length and the fecundity (Mathur, 1964; Swarup, 1961; Doha and Hye, 1970; Shafi *et al.*, 1977)

Ali *et al.*, (1994) observed sex ratio (M: F) of *Clarias batrachus* that was 1:0.857. He also observed monthly dynamics of GSI and found spawning season from April to May. There existed highly positive correlation between fish body weight and fecundity (r = 0.938), length and fecundity (r = 0.979), total weight and gonad weight (r = 0.979), ova diameter and fecundity (r = 0.852) and a weakly positive correlation between ova diameter and gonad weight (r = 0.706). The maximum fecundity was 6050.

Barua *et al.*, (1989) stated that *Clarias batrachus* has a single spawning season which last from may to July. Gonado-somatic index varies in both sexes, reaching the peak in the month of June. They also observed that the mean GSI value was the highest during the highest temperature and the highest rainfall of the year.

Hada *et. al.*, (1997) worked on sex determination of *Clarias batrachus* through external characteristics. Saha *et al.*, 1998 fed formulated diets to the larvae of *Clarias batrachus* and observed growth and survival rate. Habib and Hasan (1995) worked on the evaluation of silkworm pupae as dietary protein source for Asian catfish *Clarias batrachus* (L.) fingerling. Yasmin *et al.*, (1998) made experiment on *Clarias batrachus* by feeding live and prepared feeds and showed that live and artificial feed shows better growth performance than artificial feed only. Barua *et al.*, (1989) worked on the efficacy between

stripping and testes maceration in magur, *Clarias batrachus* on fertilization and hatching. They found that, fertilization and hatching rate increase with the increase of dose. Best fertilization and hatching rate yielded from HCG.

Khan *et al.*, (2002) reported that the fecundity of *Plotosus canius* showed linear relationship with total length, body weight and ovary weight. He also reported that the correlation regression equation of total length–fecundity, body weight–fecundity and gonad weight–fecundity were r = 0.832619, r = 0.933599 and r = 0.96118 respectively. On an average 2.3718 ova were produced in per gram body weight. Study on gonado-somatic index indicated mature fish become available from April to August, the peak being July.

Khan *et al.*, (1992) observed 139 female specimen of *Mystus tengra* collected from 12 months. The smallest and the biggest sample were found in the month of October (5cm) and May (7.7cm), respectively. Statistical analysis showed a significant correlation with length and weight (r = 0.9365428, P = 0.1). Fecundity studied during the peak-breeding season June and July varied from the minimum 720 to the maximum 733 eggs.

Sarker *et al.*, (2002) showed the fecundity and gonado somatic index of *Mystus gulio* at different length ranges. Fish from 10-20 cm showed different fecundity. Significantly highest mean fecundity was found 21589 (19394-23481) from 20-22 cm length ranges of fish. Whereas, significantly lowest mean fecundity was 11887 (11436 – 12712) from 10-12 cm length ranges of fish. Ovary weight ranges from 16 to18 gm showed significantly highest fecundity 21378. This study stated that the fecundity of fish increased with the increase in size, weight and gonad weight as well. The highest GSI values were found in the month of July. Furthermore, GSI showed that *M. gulio* has a wide spawning season. Scott (1992) found that reduced diet led to lower percentage of mature fish and lower fecundity.

Azadi *et al.*, (1987) reported that fecundity of the *Mystus vittatus* (Bloch) vary from 2515 to 9789 with a size from 89 mm to 115 mm. The fecundity was found to bear linear relationship with total length, body weight and ovary weight.

Das *et al.*, (1989) reported that the fecundity of the *Heteropneusts fossilis* ranged from 1200 to 23500, total length from 103 mm to 298 mm and body weight 7.5g to 73g with an average fecundity of 9722. The diameter of eggs ranged from 800 to 1500 microns. The fecundity of this fish bears linear relationship with total length and also with body weight.

2.3 Materials and Methods:

From the present study, a total of 124 random specimens of *Clarias batrachus* were collected monthly from May 2002 to April 2003 from the local fish market, Khulna. Live fish were firstly washed and cleaned by blotting paper to dry the specimen. Then the total length (TL) and standard length (SL) of the species were measured with the help of a centimeter scale. The total body weight was taken with the help of an electric balance (B-303S). The length and weight were recorded in the nearest centimeter and gram, respectively.

2.3.1 Sex ratio:

The fishes were dissected and then the ovary and testes were removed very carefully. In the present study, sex of every fish was ascertained by examination of gonad under microscope. The ovaries were identified by the presence of rounded ova in them and testes by the absence of any round ova in them.

The sex ratio from the collected fishes was estimated monthly. The number of male and female in percentage was plotted against months. Chi-square test (χ^2) was made to find out the statistical difference of sex, if any, from the hypothetical ratio *i.e.*, 1: 1.

2.3.2 Fecundity:

For the fecundity study, 17 female species were randomly collected from May 2002 to August 2002. The fishes were dissected and the ovary was removed very carefully. In the present study, sex of every fish was ascertained by examination of gonad under microscope. The ovaries were identified by the presence of rounded ova in them. The ovaries were preserved in 5% formalin with proper labeling to permit hardening of the ova to facilitate for subsequent studies.

Samples from anterior, middle and posterior regions of each lobe of the pair were weighed accurately. The numbers of eggs were then found out by counting with the help of a needle and magnifying glass. The mean number of eggs was then calculated in the two ovaries of each specimen. This mean number of eggs multiplied by the total weight of the two ovaries gave the total number of eggs in a particular fish.

It has been shown by Kesteven (1942) that the number of ova holds some exponential relation with the length of fish in the same way as does the weight. The relationship between fecundity- total length, fecundity- standard length, fecundity-body weight and fecundity-gonad weight were determined by the method of least square, regression lines were fitted to the data and coefficient of correlation was calculated.

The arithmetic relationship between them is as follows:

Fecundity – Total length: $F = a + b\ TL$
Fecundity – Standard length: $F = a + b\ SL$
Fecundity – Body weight: $F = a + b\ BW$
Fecundity – Gonad weight: $F = a + b\ GW$

The logarithmic relationships between them are as follows:

Fecundity – Total length: $\log F = \text{Log } a + b \text{ Log } TL$
Fecundity – Standard length: $\log F = \text{Log } a + b \text{ Log } SL$
Fecundity – Body weight: $\log F = \text{Log } a + b \text{ Log } BW$
Fecundity – Gonad weight: $\log F = \text{Log } a + b \text{ Log } GW$

2.3.3 Gonado Somatic Index (GSI):

The gonadosomatic index of the collected samples was calculated for males and females separately. The fishes were dissected and the ovary and testes were removed very carefully. In the present experiment, sex of every fish was ascertained by examination of gonad under microscope. The weight of each ovary and testes were taken with the help of electric balance (B-303S) and calculated according to the following equation cited by Parameswarn *et al.*, (1974). This is more suitable to explain the maturity of fishes.

$$GSI = \frac{W_1}{W_2} \times 100$$

Where, W_1 = wet weight of gonad

W_2 = total wet weight of fish (male or female)

2.4 Results

2.4.01 Sex ratio:

In the present experiment, overall sex ratio of *Clarias batrachus* was found 1: 0.94 (Male: Female). Similar sex ratio (1: 1) of male over female was observed in the month of March, May, August and September. Lower ratio of male over female was found during the month of June, July and October. Higher ratio (M: F) was observed in the month of January, February, April, November and December. The highest and lowest sex ratios were found in the month December (1:0.67) and July (1:1.25) respectively (Table:1 and Fig.1). The sex of male to female was found to be homogenously distributed *i. e.*, 1:1 ratio at 5% level of significance that was analysed by chi-square test.

Table 1: Sex Ratio of *Clarias batrachus*

Month	No, of fishes	Male	Female	% of Male	% of Female	M: F
May	10	5	5	50.00	50.00	1:1
June	11	5	6	45.45	54.55	1:1.20
July	9	4	5	44.44	55.56	1:1.25
August	10	5	5	50.00	50.00	1:1
September	10	5	5	50.00	50.00	1:1
October	13	6	7	46.15	53.85	1:1.17
November	9	5	4	55.56	44.44	1:0.80
December	10	6	4	60.00	40.00	1:0.67
January	12	7	5	58.33	41.67	1:0.71
February	9	5	4	55.56	44.44	1:0.80
March	10	5	5	50.00	50.00	1:1
April	11	6	5	54.55	45.45	1:0.83
Total	124	64	60			1:0.94

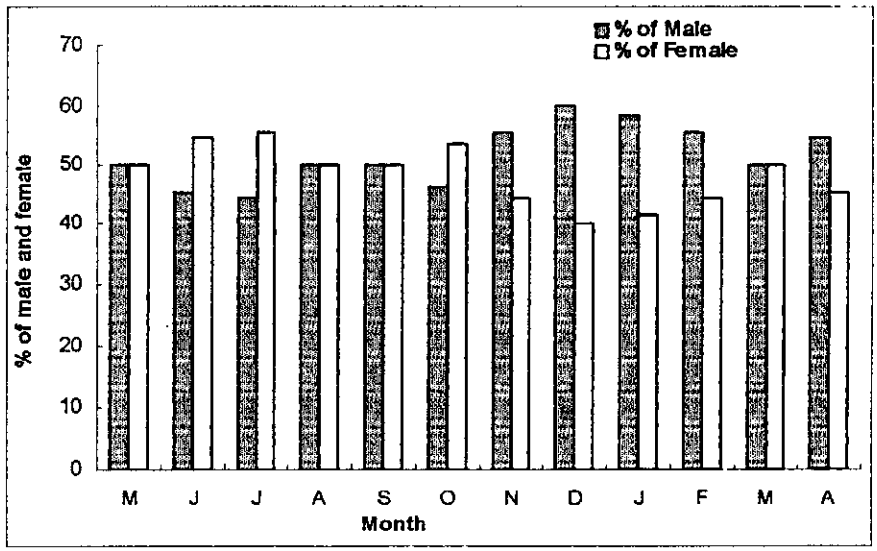

Fig 1: Sex ratio of *Clarias batrachus*

2.4.02 Gonado-somatic Index (GSI):

For the male *Clarias batrachus* the highest GSI value (0.543 ± 0.09) was found in the month June. The lowest value (0.057 ± 0.019) was observed in the month December. The highest value of GSI was 19.330 ± 2.229 for female and it was found at same time as the male. The lowest GSI value (0.187 ± 0.079) for female was observed in the month September (Table: 2 and 3; Fig. 2 and 3).

Table 2: Mean GSI of male of *Clarias batrachus.*

Month	GSI ± SD
M	0.339 ± 0.042
J	0.543 ± 0.090
J	0.378 ± 0.057
A	0.337 ± 0.121
S	0.073 ± 0.010
O	0.153 ± 0.061
N	0.060 ± 0.012
D	0.057 ± 0.019
J	0.105 ± 0.011
F	0.136 ± 0.049
M	0.093 ± 0.021
A	0.192 ± 0.058

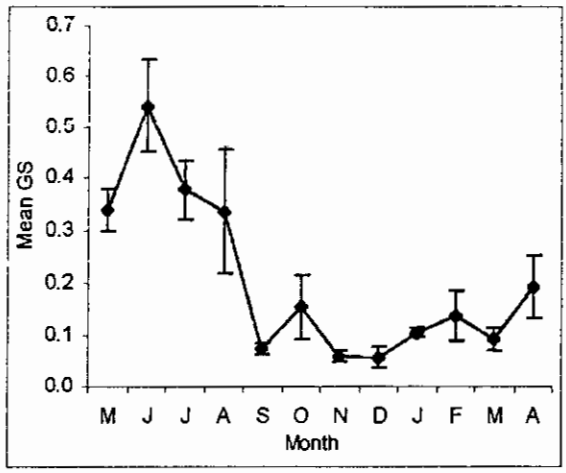

Fig 2: Mean GSI of male of *Clarias batrachus*

Table 3: Mean GSI of female of *Clarias batrachus*

Month	GSI ± SD
M	8.250 ± 3.087
J	19.330 ± 2.229
J	9.462 ± 3.967
A	14.525 ± 4.149
S	0.187 ± 0.079
O	0.339 ± 0.149
N	0.884 ± 0.618
D	0.578 ± 0.202
J	0.761 ± 0.154
F	1.829 + 0.317
M	2.261 ± 0.948
A	2.600 ± 2.799

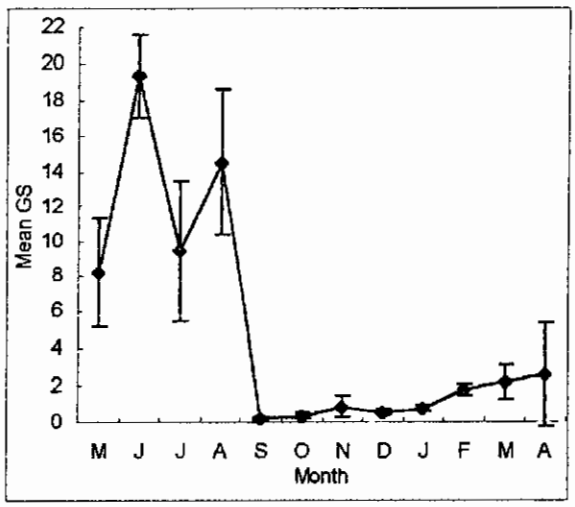

Fig 3: Mean GSI of female of *Clarias batrachus*

2.4.03 Fecundity:

The number of eggs ranged from 20970 to 1875 approximately. The maximum number of eggs was counted in a sample with 32.5 cm in total length and 280.5 gm of total body weight whereas minimum number of eggs was found in a sample measuring 22.3 cm in total length and 77.477 gm in total body weight (Table: 4).

Table 4: Fecundity of *Clarias batrachus*

TL	SL	TBW	GW	Fecundity	Log TL	Log SL	Log TBW	Log GW	Log fecundity
23.5	20.8	91.225	6.74	3362	1.318	1.318	1.960	0.829	3.527
22.1	19.6	84.34	6.681	3650	1.292	1.292	1.926	0.825	3.562
22	19.6	82.925	9.756	8478	1.292	1.292	1.919	0.989	3.928
24.1	21.3	95.801	9.982	6520	1.328	1.328	1.981	0.999	3.814
22.3	19.8	77.472	2.946	1875	1.297	1.297	1.889	0.469	3.273
27.3	24.5	171.95	33.454	16727	1.389	1.389	2.235	1.524	4.223
32.5	29.3	280.5	48.155	20970	1.467	1.467	2.448	1.683	4.322
26.7	24.1	165.78	37.743	18311	1.382	1.382	2.220	1.577	4.263
26	23	111.493	3.589	3280	1.362	1.362	2.047	0.555	3.516
24.6	22.1	108.699	14.864	14680	1.344	1.344	2.036	1.172	4.167
24.7	22.2	107.808	8.061	8250	1.346	1.346	2.033	0.906	3.916
23.7	21.8	81.025	12.425	10798	1.338	1.338	1.909	1.094	4.033
21.2	18.8	72.447	14.544	7460	1.274	1.274	1.860	1.163	3.873
22.6	19.9	70.17	12.07	6542	1.299	1.299	1.846	1.082	3.816
21.5	18.8	72.125	9.125	5221	1.274	1.274	1.858	0.960	3.718
23.5	21.6	77.126	10.215	5600	1.334	1.334	1.887	1.009	3.748
20.8	18.3	65.321	6.173	3240	1.262	1.262	1.815	0.790	3.511

2.4.04 Relationship between total length and fecundity:

The results of t test between total length and fecundity showed a linear and high degree of positive correlation at 5% level of significance for 16 degree of freedom (Figure: 4). The regression equation, values of r and t are shown below:

$Y = a + b\,TL$

Therefore $Y = -29046 + 1561.3TL$ ----------(i)

$r = 0.774$ $t = 8.604$

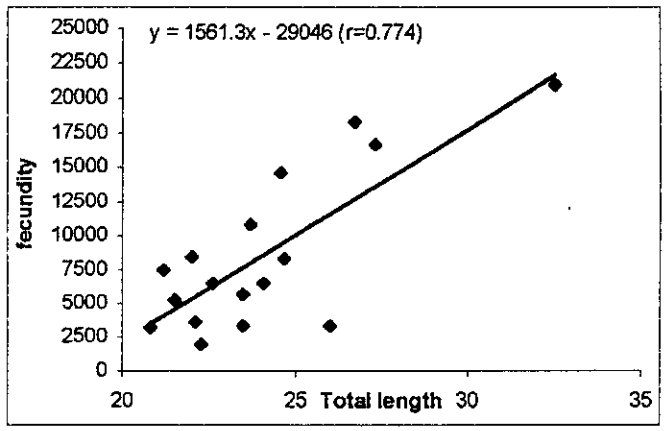

Fig 4: Arithmetic relationship between total length and fecundity

2.4.05 Logarithmic relationship between total length and fecundity:

The logarithmic relationship between total length and fecundity showed a linear and positive correlation which was significant at 5% level of significance for 16 degree of freedom (Fig. 5). The regression equation and value of r yielded from Log total length and Log fecundity are given below:

$$\text{Log } Y = \text{Log } a + b \log TL$$

Therefore $\text{Log } Y = -1.6486 + 3.978 \log TL$ ----------(ii)

$r = 0.6412, t = 4.218$

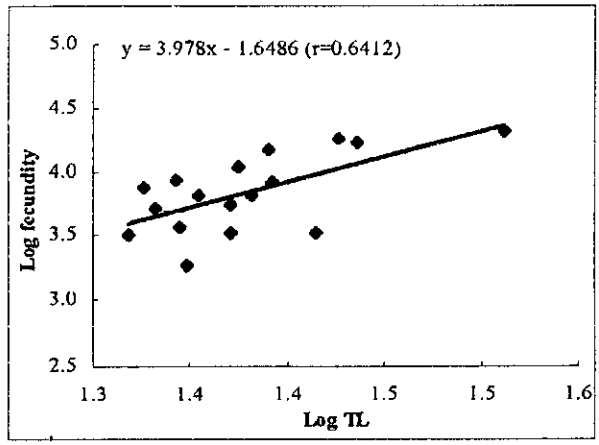

Fig 5: Logarithmic relationship between log total length and log fecundity

2.4.06 Standard length and fecundity relationship:

The arithmetic relationship between standard length and fecundity showed a linear and positive correlation which was significant at 5% level of significance for 16 degree of freedom (Fig. 6).

The regression of standard length and fecundity yielded the following equation in the arithmetic scale:

$$Y = a + b\,SL$$

Therefore $Y = -27914 + 1694.9SL$ ----------(iii)

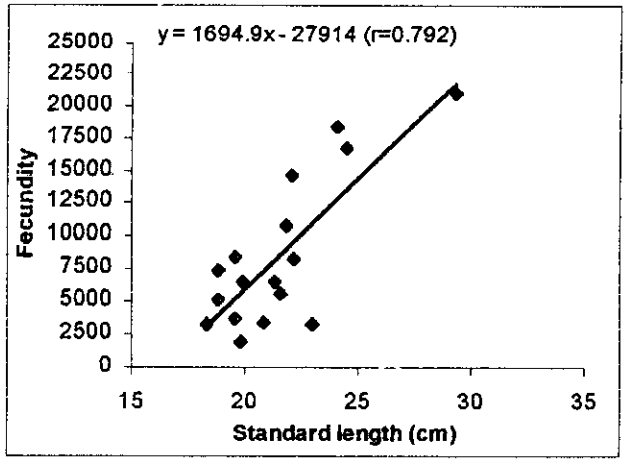

Fig 6: Arithmetic relationship between standard length and fecundity

2.4.07 Logarithmic relationship between standard length and fecundity:

Plotting Log standard length and Log fecundity, showed a linear and positive regression line. Correlation of coefficient was significant at 5% level of significance for 16 degree of freedom (Fig. 7). The regression equation and value of r are shown as bellow:

$$\text{Log } Y = \text{Log } a + b \log SL$$

Therefore $\text{Log } Y = -1.3489 + 3.899 \log SL$ ----------(iv)

$r = 0.6664$, $t = 4.467$

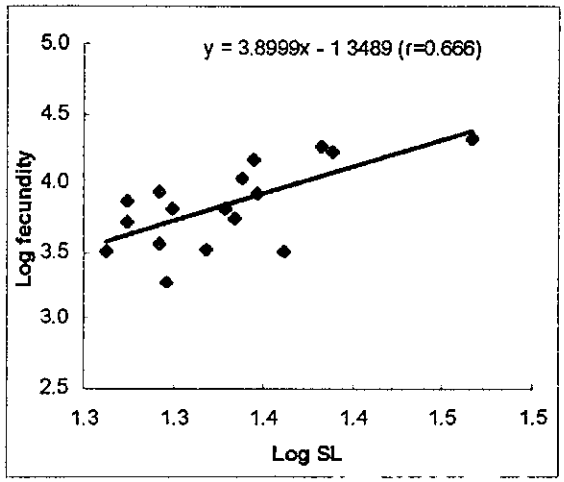

Fig 7: Logarithmic relationship between log standard length and log fecundity

2.4.08 Relationship between total body weight and fecundity:

The arithmetic between total body weight and fecundity showed a linear and positive correlation, which was significant at 5% level of significance for 16 degrees of freedom (Fig. 8) and can be regressed in the regression line as:

$$Y = a + b\,TBW$$

Therefore $Y = -840.17 + 87.681\,TBW$ ----------(v)

$r = 0.820, t = 8.851$

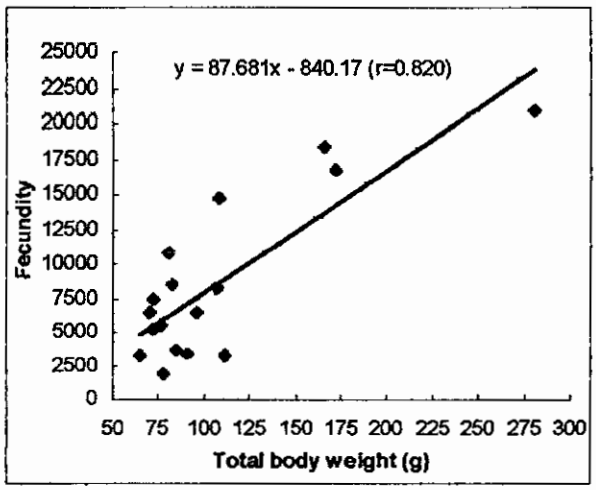

Fig 8: Arithmetic relationship between total body weight and fecundity

2.4.09 Logarithmic relationship between total body weight and fecundity:

The Log value of total body weight and fecundity showed linear and positive correlation (Fig.9). Correlation of coefficient was significant at 5% level of significance for 16 degree of freedom.

The regression equation and value of r are as bellow:

$$Y = a + b\,TBW$$

Therefore $\log Y = 1.3872 + 1.229 \log TBW$ ----------(vi)

$r = 0.6865, t = 3.657$

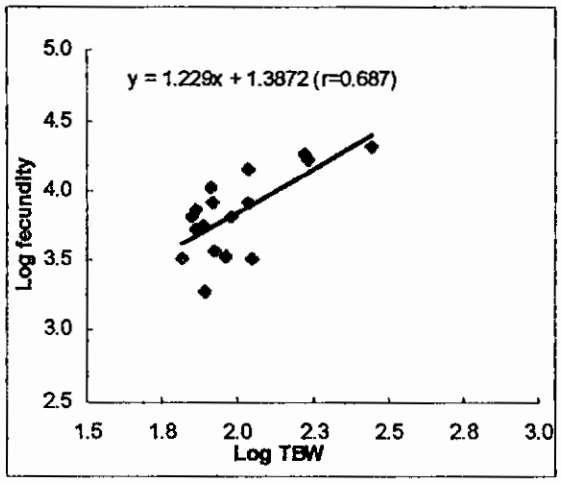

Fig 9: Logarithmic relationship between log total body weight and log fecundity

2.4.10 Relationship between gonad weight and fecundity:

The arithmetic relationship between gonad weight and fecundity showed a linear and positive correlation which was significant at 5% level of significance for 16 degree of freedom (Fig.10). And can be regressed in regression line as:

$$Y = a + b\,GW$$

Therefore $Y = 2423.7 + 420.9\,GW$ ----------(vii)

$r = 0.927, t = 8.336$

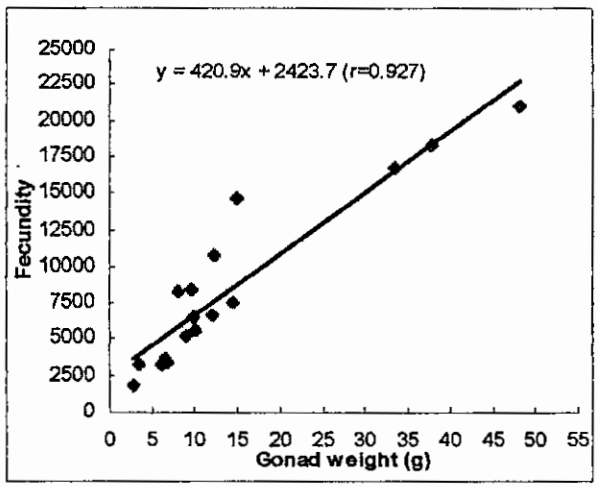

Fig 10: Arithmetic relationship between gonad weight and fecundity

2.4.11 Logarithmic relationship between gonad weight and fecundity:

The relationship of Log gonad weight and Log fecundity showed a linear and positive correlation and coefficient of correlation was significant at 5% level of significance for 16 degree of freedom (Fig.11).

The regression equation can be expressed as:

$$Y = a + b\ GW$$

Therefore $\text{Log } Y = 2.9499 + 0.8544 \log GW$ ----------(viii)

$r = 0.9291, t = 8.991$

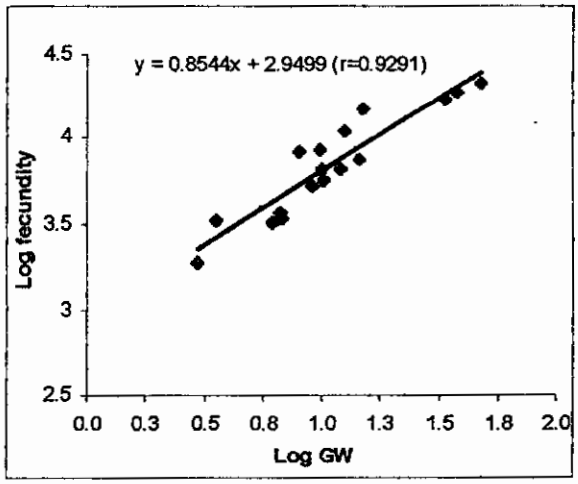

Fig 11: Logarithmic relationship between log gonad weight and log fecundity

2.5 Discussion:

2.5.1 Sex ratio:

The present experiment showed slightly disproportionate occurrence of the number of two sexes of *Clarias batrachus* (Male to female = 1: 0.94). From Table 1 it was found that the sex ratio in the month of May, August, September and March was equal to the hypothetical sex ratio (1:1). The occurrence of male was higher than female in the month November, December, January, February and April. But the occurrence of female was higher than male in the month June, July and October. This variation may probably be due to fishing and abundance of the particular sexes. Ali *et al.*, (1994) observed sex ratio (M: F) of *Clarias batrachus* that was 1:0.857 that agrees with the ratio of the present study.

2.5.2 Fecundity:

The fecundity of *Clarias batrachus* ranged from 1875 to 20970. The maximum fecundity was found in the fish with total length 32.5 cm, total body weight 280.5g and gonad weight 48.155g. The average fecundity was observed 8527 (Table 4). Ali *et al.*, (1994) reported that the maximum fecundity of *Clarias batrachus* was 6050 that disagrees with the result of the present study.

In the present investigation, a linear and positive relationship was found between fecundity and total length. This agrees with the result of Ali *et al.*, (1994) on the same species. From the present study it is evident that a linear relationship exists between fecundity and standard length. A positive relationship between fecundity and body weight was observed in the experiment that supports with the result done by Ali *et al.*, (1994). In the current experiment a positive linear relationship between fecundity and gonad weight aggress with the result of Ali *et al.*, (1994).

A comparison of correlation of total length-fecundity (r=0.774), standard-fecundity (r = 0.792), body weight-fecundity (r = 0.820) and gonad weight-fecundity (0.927) indicate a much closer relationship of fecundity with gonad weight than the other factors. Present investigations agree with the results of Rahman (1989) on *Mystus gulio*. Ali *et al.*, (1994) observed there existed highly positive correlation between fish body weight and fecundity (r =0.938), length and fecundity (r =0.979), total weight and gonad weight (r = 0.979).

In the present experiment all the t values for different relationship were higher than the tabulated values at 5% level of significance.

2.5.3 Gonado-somatic Index (GSI):

The highest mean value of GSI was found in the month of June for both male and female. The maximum GSI value of male and female were 0.543 and 19.330 respectively. The lowest mean value of GSI (0.057) of male was found in the month of December and the lowest value (0.187) for male was found in September.

Monthly average GSI values of male are graphically presented in figure 2. From the figure it is evident that GSI values were found to increase from April onwards reaching a peak in June followed by a gradual decrease up to August. A sudden drop was observed in the month September and the GSI values were very poor up to March. Increases in GSI values of male indicated development of gonads during April to June. The same results also found in case of female (Figure 3). Increases in GSI values of female indicated development of gonads during April to June. Sudden drop of GSI values in September indicated spent stage of fish. From this information, it can be noted that the spawning season of *Clarias batrachus* is from April to August with the peak season in the month of June. Ali *et al.*, (1994) observed monthly dynamics of GSI and found spawning season from April to May with the peak season in April for *Clarias batrachus*. This result is nearly closer with the result of the present experiment. Barua *et al.*, (1986) stated that *Clarias batrachus* has a single spawning season which last from may to July. Gonado-somatic index varies in both sexes, reaching the peak in the month of June. His results completely agrees with the results of the current study. The slightly variation of GSI in the present study in comparison with others could be due to habitat differentiation, food availability and temperature fluctuation.

Biochemical Composition

3.1 Introduction

Increase in population is creating an alarming situation in terms of 'protein deficiency' and becoming a major hazard in developing countries. Almost 75% peoples of Bangladesh have been suffering from malnutrition. In this country, per capita annual fish needed 18 Kg but per capita annual fish intake only 12 Kg (DoF, 2001). To overcome this problem it is necessary to supply protein rich diet to the people. Fish is a major source of animal protein. Biochemical composition analysis is an index of nutritive value as isolated fractions are correlated with some of the properties of organisms that are nutritionally significant. It provides both quantitative and qualitative information on the uncertain nutritional significance of a particular organism of feed stuff, which are essential for both nutritionalist and to the fishery technologist and it is also essential for the maximum utilization of natural resource.

Biochemistry concerns with the chemical reaction of living organism. Protein, Lipid, Moisture and Ash are the major biochemical constituents of organism. These can be affected by a numerous factors. The chemical composition of fish expected to vary largely from one species to another and with in the same species from one individual to another individual and even in the same species. The composition of a fish may vary due to location, size, age, sex etc. composition may also vary from one part to another part (Stansby, 1962).

Now a days fish has been widely accepted by the both urban and rural people throughout the world. Biological or nutritional quality of protein contained in various fish species compare very favorable than that of muscle meat. A special advantage of fish is the highest degree of digestibility in comparison of others. The constituents (protein, fat, moisture, ash) play vital role in evaluating the quality of fish. Fish protein contains favorable amount of all essential amino acids. Fish have been favored in the diet because of high content of essential fatty acids.

Extensive work on biochemical composition of many fishes have been done in our country (Hossain *et al.*, 1999). But few reports have been published (Hossain *et al.*, 1999 and

Rahman *et al.*, 2003) on biochemical composition of *Clarias batrachus*. Their results revealed only the body flesh in one month rather than monthly variation and other body parts.

Most of the people in our country are ignore about the quality of their daily food intake. They have no idea about the nutrient content of fish. On the basis of the above discussion, the investigation on *Clarias batrachus* has been focused under the following objectives:

- To estimate the annual nutrient content in edible part of the body of both male and female.
- To determine the nutrient content in the whole body.
- To access the nutrient value at different parts of the fish.

3.2 Review of literature:

Prior to conduct an experiment, it is essential to know the information about the previous related works. Considerable information regarding the biochemical composition of various fishes is available but very little is known about the biochemical composition of Magur (*Clarias batrachus*). The literature cited here, will therefore, include information about other fishes, which are relevant to the present study.

Chemical composition of fish are affected by numerous factors such as sexual stage of development (Legendre, 1983), species diversification (Jaquot, 1961) and difference in age (Khuda *et al.*, 1962).

Stansby (1962) reported a large variation in proximate composition of fish from species to species and also among fishes of the same species. For the whole fish, he reported range of moisture, protein, fat and ash content was 28 to 90%, 6 to 28%, 0.2 to 64% and 0.4 to 1.5%, respectively. He also reported that the average value of moisture, protein, fat and ash content were 74.8%, 19%, 5% and 1.2%, respectively.

Chowdhury (1981) worked on the chemical composition and nutritive quality of some fresh water zeolfish of Bangladesh. He determined protein, moisture, fat and ash content of large, medium and small size of *Clarias batrachus and Heteropneustes fossilis.* He found the maximum protein and moisture content in small size and the maximum fat and ash content in large size of *Clarias batrachus.* . He also observed that incase of *Heteropneustes fossilis* the maximum protein, fat and ash content were in large fish and the maximum moisture was in medium size fish.

Puwastien *et al.*, (1999) studied on proximate composition of *Clarias macrocephalus.* He found the protein moisture, fat and ash contents as 71.6%, 17.0%, 8.9% and 1.0% respectively.

Saha and Guha (1939, 1940) studied 34 species of fresh water fishes of Bangladesh in regard to their nutritive values. They found that the cat fish (*H. fossilis*) had the highest protein content (22.8%). The major carps (*C. catla, C. mrigala, L. rohita, and L. calbasu*)

contained 19.5%, 19.55, 16.6%, and 14.7% protein respectively in their flesh. Hilsha was the richest source of fat followed by *S. silondia, P. pangasius, B. sarana and A. testudineus* with values 12.1%, 10.8%, 9.9% and 8.8%, respectively. Rahman *et al.*,(1978) worked on the biochemical composition of *L. rohita* and found that the raw whole fish had moisture, protein, lipid and ash were 70.9%, 19%, 5.1% and 2.42%, respectively.

Khuda *et al.*, (1962) conducted a biochemical and nutritive value of some native species. Their data showed that shoal (*Channa striatus*), lata (*Ophiocephalus punctatus*) and shingi (*Heteropneustes fossilis*) contained respectively 75.83%, 75.85% and 77.92% moisture 18.41%, 20.1% and 19.61% protein, 0.64%, 1.08% and 1.23% fat, 1.15% , 1.36% and 1.33% ash.

Mowlah (1976) investigated that the protein content of *Hilsa ilisha* decreases from 21.26% - 17.44% with a simultaneous increase in moisture content from 62.14% - 71.90% during storage period in ice.

Azim (1982) found 3.08%, 2.93% and 0.85% fat content in fresh *Pampus argenteus, Hilsa ilisha* and *Penaeus monodon*, respectively.

Ahmed *et al.*, (1984) evaluated 5.30% to 9.50% moisture content in commercially important fresh water dry fish. Mannan (1977) found 49.5% moisture in, *Hilsa ilisha*.

Hossain *et al.*, (1999) was analyzed on proximate composition of 23 small indigenous fish species and reported that the moisture content of different species ranged between 71 and 81.94%. The muscle protein content among the species varied widely (16.16-22.28%). The carcass lipid content varied between 1.87 and 9.55%.

Berg *et.al.*, (1998) reported that fat, protein and specific energy declined greatly in winter but was replenished rapidly in spring. Rates of decline were slower for the smallest fish, which also had the lowest specific content of fat, protein and energy, while the differences in absolute amounts were greatest for the largest fish.

Kamal *et al.*, (2000) studied on the post mortem changes in shrimp and prawn during ice storage: ii. Biochemical aspects of quality changes and showed that at the end of 10 days of ice storage, moisture and protein content of fresh water prawn slightly decreased from 78.34 to 77.35 and 18.46 to 17.10, respectively, while lipid and ash content slightly increased. The moisture, crude protein, lipid and ash content of one day ice stored tiger shrimp samples were 78.07, 18.06, 1.3 and1.29%, respectively.

3.3 Materials and Methods:

For the present study, a total of 100 random adult specimens of *Clarias batrachus* were collected monthly during May 2002 to April 2003 from local market, Khulna. Among them 50 were male and 50 were female. The experiment was conducted in the Fish Nutrition Laboratory of Fisheries and Marine Resources Technology Discipline, Khulna University, Khulna. Fishes were firstly brought in the laboratory and then cleaned with blotting paper. The total length and standard length of the species were measured with the help of a centimeter scale. The total body weight was taken with the help of an electric balance (B-303S). The length and weight was recorded in the nearest centimeter and gram respectively.

Biochemical composition:

Whole body and flesh (wet basis)

Biochemical composition body flesh and whole body of *Clarias batrachus* was determined. Composition of body flesh was determined in wet basis. In case of whole fish biochemical composition was done in dry basis. For body flesh, flesh was taken from three male and three female species separately. The percentage of biochemical composition (protein, fat, ash and moisture) was determined for each species. Then the mean percent value of each biochemical composition of male and female was calculated separately.

In case of whole fish, two male and two female were taken and weighed. Then the fishes were placed in an oven (N4OC) at 105^0C for 24 hrs. After drying they were weighed again and data were recorded.. The percentage of biochemical composition (protein, fat, ash and moisture) was determined for each species. Then the result was converted into wet basis. For each experiment three replications were done. Monthly experimental work was done round the year.

Body parts:

Biochemical composition of different body parts (Head, Middle and Tail) was determined only in the month of July. In case of body part, head, middle and tail portion of both male and female were separated with scalpel. Three specimen from each sex were taken. The

biochemical composition of separate parts was determined in dry basis same as whole fish. Three replications for each specimen were done.

3.3.1 Protein:

Principle:

The protein of the fish body was broken down and the amount of free Nitrogen was determined. The amount of protein was determined by multiplying the value of Nitrogen with a constant. Under mentioned apparatus were used to conduct the experiment.

- Kjeldahl flask.
- Kjeldahl nitrogen digesting apparatus (NDG-50E).
- Kjedhal nitrogen distillation apparatus (OSK-8416 A).
- Burette.
- Electric balance (B303 S).
- Blender.
- Conical flask.
- Measuring cylinder.
- Mortar

Reagents and its preparation:

1. Resolvent: Potasium sulphate and murcuric oxyde in the ratio of 10 to 0.7 were mixed and crushed properly and preserved in colored bottle.
2. Concentrated sulfuric acid was taken for the study.
3. 2% Boric acid solution. 20 gm Boric acid crystal was added with 1 liter distilled water.
4. Tashiro's indicator .dissolve 80 gm methyl red and 20 mg methylene blue in 95 % ethanol and make up to 100 ml with 95 % ethanol .
5. Sandy zinc
6. 33-40% Caustic soda, 400 gm NaOH and 10 gm potassium sulfied was added with 1 liter distilled water.
7. 0.1 N HCl Acid. 8.48 ml HCl was added with 1 liter distilled water.

Procedure:

Sample preparation:

Incase of body flesh, the flesh of fish was blended with blender machine and then the sample was taken. For whole fish, the whole body of the fish was completely dried in an oven at 105^0 C. Then the dried fish done dust with a morter and then the sample was taken for experiment.

Digestion:

0.2-0.5 g of sample was weighed and inserted into a Kjeldahl flask and two g of resolvent and 5ml concentrated H_2SO_4 were added into the flask. The content of the flask was digested by heating in a Kjeldahl nitrogen digesting apparatus (NDG-50E) for 45 minutes till the clear color appears. After completion of digestion, transferred flask from digesting apparatus and let it be cooled for 10 minutes until the temperature decreased upto about room temperature.

Distillation:

15 ml of 2% Boric acid (H_3BO_3) were taken in a conical flask and 2-3 drops of Tashiro's indicator were added into the flask. The delivery tube of the apparatus was arranged with its tip below the surface of 15 ml boric acid. Than the digested materials were diluted by adding 70 ml of distilled water and 0.5 g of sandy zinc was added in the Kjeldahl flask. About 25 ml of 33-40 % Caustic Soda (NaOH) solution was poured slowly along the flask's in side wall . the flask was than connected to the Kjedhal nitrogen distillation apparatus (OSK-8416 A) and distilled it for about 30 minutes to obtain 25 –30 ml of distilled solution . Distilled solution was stored in the conical flask through delivery tube.

Titration:

The distilled solution stored in the conical flask was taken and titrated against 0.1 N HCl solution.

Calculation:

The percentage of gross proteinous nitrogen was calculated out with the following formula (Crampton and Harris , 1969; Mitchell ,1972; Jacobs ,1973 ;Pearson ,1976):

$$\%N = \frac{\text{Volume of HCl X Normality of HCl X } 0.014}{\text{Weight of Sample (g)}} \times 100$$

Percentage of protein = %N X 6.25 (Conversion factor)

3.3.2 Lipid

Principle

Fat is extracted with acetone by Soxhlet extraction and the extract thus obtained is weighed after careful recovery of the solvent. The following apparatus were used to conduct the experiment.

- Soxhlet fat extractor (SF-6)
- Extracting thimble paper
- Hot plate (SH-1)
- Electric balance (B303 S).
- Blender.
- Beaker (250 ml).
- Mortar

Chemical :

- Acetone

Procedure:

Incase of body flesh, the flesh of fish was blended with blender and then the sample was taken.. For whole fish, the whole body of the fish was completely dried in an oven at 105^0 C. temperature. Then the dried fish done dust with a mortar and then the sample was taken for experiment.

2-5 gm sample was taken in the thimble paper.First of all weight of the empty thimble paper was taken. Than it was weighed with the sample. Difference between two weights gave the weight of the sample. Than the sample was placed in soxhlet fat extractor apparatus. Fat extracted with acetone was taken in a weighed 250 ml beaker and the it was dried with hot plate at 105^0 C. temperature and finally weighed. Fat weight was found from the difference between two weights.

Calculation:

Calculation was done by using the following formula (Maynard, 1970; Jacobs, 1973 & Pearson, 1976)

$$\% \text{ Fat} = \frac{\text{Weight of extracted fat (g)}}{\text{Weight of sample (g)}} \times 100$$

3.3.3 Moisture

Principle

Moisture is determined by complete drying of the sample at 100 5^0 C. The loss in weight of the sample is the measurement of moisture content. Under mentioned apparatus were used to conduct the experiment.

- Oven (N40C)
- Porcelain crucibles
- Electric balance (B303 S)
- Blender.

Procedure:

For determining moisture a porcelain crucible was cleaned, dried and then the constant weight of the crucible was taken. Sample was placed in the crucible and weight was taken. Difference between two results gave the weight of the sample. Then the crucible with sample was put in a controlled oven and was dried at 105 ^{0} C. and was weighed several times till the constant weight was achieved

Calculation:

The percentage of moisture content was calculated by the following formula (Pearson, 1976).

$$\% \text{ Moisture} = \frac{\text{Weight of the sample (g) - weight of the dried sample (g)}}{\text{Weight of the sample (g)}} \times 100$$

3.3.4 Ash:

Principle

The ash content is the inorganic residue let after complete removal of the organic residue by muffling at about 550 – 600 ^{0}C in the muffle furnace. The following apparatus were used to conduct the experiment.

- Muffle furnace (S-336 RB)
- Porcelain crucible and desiccators.
- Electric balance (B303 S).
- Blender.
- Mortar

Procedure:

The flesh of fish was blended with blender Incase of body flesh and then the sample was taken. For whole fish, the whole body of the fish was completely dried in an oven at 105^0 C. temperature. Then the dried fish done dust with a mortar and then the sample was taken for experiment.

Cleaned porcelain crucible were heated in a muffle furnace 600 ^{0} C and the crucible were cooled and weight, heating, cooling and weighing are repeated until constant weights of the crucible were obtained. The sample with the crucible was weighed and recorded. 2 to 5 gm of sample was taken in each crucible. After that; each crucible with the sample in the muffled furnace. The sample were muffled at 600 0 C for 6 hrs. Until the color of the residue become uniformly grayish to white. Afterwards the crucibles were transferred to the desiccators to cool down at room temperature for a few minutes. Heating desiccating and weighting were repeated till a constant weight obtained. Finally the constant weight of the crucibles was recorded.

Calculation:

The percentage of ash content was determined by the following formula (Mayard, 1970 and Pearson, 1976).

$$\% \text{ Ash} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

3.4 Result

3.4.1 Moisture:

The moisture content was (as percent) found monthly in body flesh and whole body of both sexes of *Clarias batrachus* is shown in the Table: 5.

Body flesh: The highest moisture content was observed in the month of July and September for male (80.01%) and female (80.12%), respectively. The minimum moisture content for male (75.03%) and female (75.87%) was found in the month of December and January accordingly. The average moisture content of body flesh of male and female was 77.92% and 78.20%, respectively.

Whole body: The highest moisture content of male (79.13%) and female (76.72%) was found in July and October respectively and the lowest value was calculated in May for both sexes (male 71.99% and female 72.69%). The average moisture content of whole fish of male and female was 75.35% and 75.36% respectively.

Table 5: Monthly average moisture content of *Clarias batrachus*.

Month	Male		Female	
	Body flesh	Whole fish	Body flesh	Whole fish
May	77.62 ± 1.230	71.99 ± 1.761	76.19 ± 1.341	72.69 ± 0.723
June	77.33 ± 1.441	74.38 ± 0.354	79.34 ± 3.724	74.78 ± 0.590
July	80.01 ± 0.085	79.13 ± 0.163	79.56 ± 1.303	75.85 ± 0.216
August	79.59 ± 1.301	77.33 ± 0.120	80.01 ± 0.088	76.59 ± 0.150
September	79.66 ± 1.902	75.72 ± 0.125	80.12 ± 1.175	76.32 ± 0.116
October	78.66 ± 3.511	72.84 ± 0.118	78.13 ± 1.564	76.72 ± 0.276
November	77.94 ± 0.460	74.55 ± 0.610	77.99 ± 0.611	75.42 ± 0.291
December	75.03 ± 1.853	75.19 ± 0.062	76.22 ± 0.210	75.54 ± 0.420
January	76.14 ± 0.112	76.39 ± 0.659	75.87 ± 0.483	76.13 ± 0.140
February	75.68 ± 0.954	75.41 ± 0.318	76.73 ± 0.821	75.75 ± 0.354
March	78.07 ± 0.196	75.73 ± 0.170	77.92 ± 0.284	75.02 ± 0.147
April	79.34 ± 0.531	75.52 ± 0.129	80.33 ± 0.129	73.56 ± 0.621
Mean	77.92 ± 1.128	75.35 ± 0.383	78.20 ± 0.974	75.36 ± 0.338

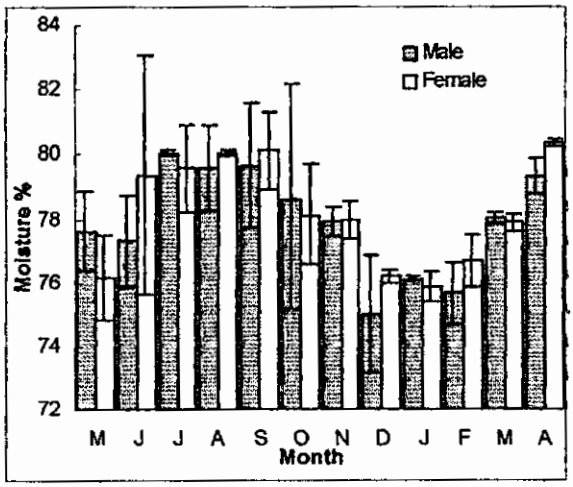

Fig 12: Monthly variation of moisture % of body flesh of *Clarias batrachus*. Error bar indicates SD

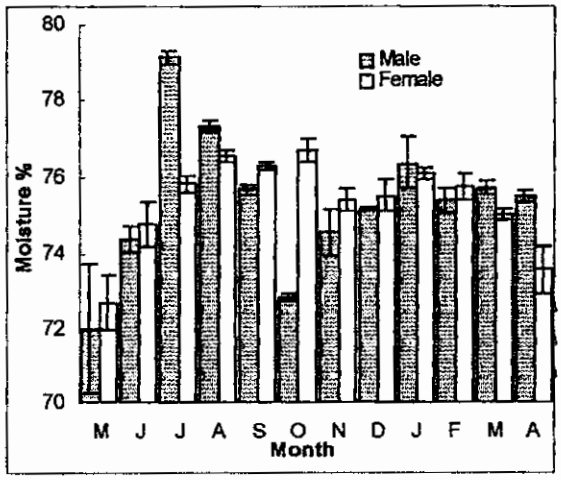

Fig 13: Monthly variation of moisture % of whole body of *Clarias batrachus*. Error bar indicates SD

Different body part: The maximum moisture content (80.43%) was observed in the middle of female and the minimum value (69.87%) was found in the head portion of male (Table. 6)

Table 6: Moisture content of different body parts of *Clarias batrachus*.

Body Part	Female (%)±SD	Male (%)±SD
Head	71.90 ± 0.136	69.87 ± 0.270
Middle	80.43 ± 0.150	79.31 ± 0.238
Tail	80.00 ± 0.185	78.70 ± 0.223

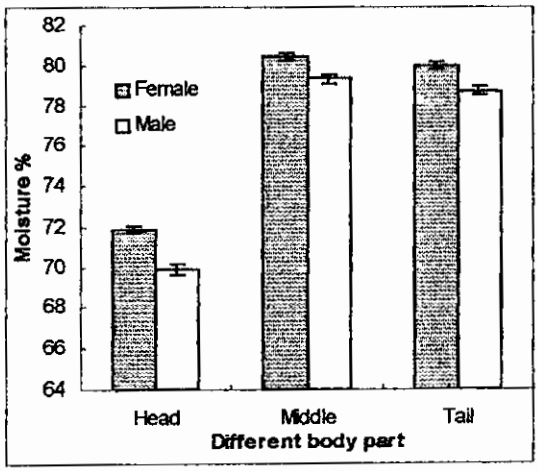

Fig 14: Moisture content of different body parts of *Clarias batrachus*. Error bar indicates SD

3.4.2 Protein:

The protein content (as percent) observed monthly in body flesh and whole body of male and female of *Clarias batrachus* is presented in the Table: 7.

Body flesh: The maximum protein content for male (21.75%) and female (21.35%) was found in the month October and September respectively. The minimum value was calculated in July for male (18.455) and in December for female (18.62%). The average protein content of body flesh of male and female was 19.67% and 19.47% respectively.

Whole body: The maximum protein content was observed in the month of December and July for male (19.32%) and female (18.90%) accordingly. The lowest value for male (15.82%) and female (15.25%) was found in the month of July and May respectively. The average protein content of whole body of male and female was 17.44% and 17.47% respectively.

Table 7: Monthly average protein content of Clarias batrachus

Month	Male		Female	
	Body flesh	Whole fish	Body flesh	Whole fish
May	18.70 ± 0.244	15.97 ± 0.049	18.684 ± 1.444	15.2525 ± 0.521
June	20.76 ± 0.663	16.821 ± 0.283	19.2065 ± 2.879	17.449 ± 0.946
July	18.45 ± 1.194	15.822 ± 0.182	18.8315 ± 0.591	18.899 ± 0.154
August	20.19 ± 0.582	15.924 ± 0.065	20.303 ± 0.040	18.0445 ± 0.203
September	21.41 ± 2.380	18.142 ± 0.206	21.35 ± 1.192	18.8065 ± 0.839
October	21.75 ± 3.897	16.422 ± 0.879	19.855 ± 1.387	17.828 ± 1.916
November	19.42 ± 0.296	17.182 ± 0.079	19.6295 ± 0.677	18.292 ± 0.465
December	19.165 ± 0.761	19.322 ± 0.901	18.6245 ± 3.201	18.213 ± 0.294
January	19.03 ± 0.150	18.0615 ± 0.090	19.3445 ± 0.311	18.5035 ± 0.192
February	19.54 ± 0.268	18.554 ± 0.195	19.019 ± 0.177	18.325 ± 0.286
March	19.06 ± 0.090	18.2895 ± 0.231	19.8255 ± 0.260	18.8225 ± 0.260
April	18.59 ± 0.540	18.75 ± 0.300	19.016 ± 1.570	18.4945 ± 0.179
Mean	19.67 ± 0.919	17.44 ± 0.372	19.47 ± 1.140	18.08 ± 0.521

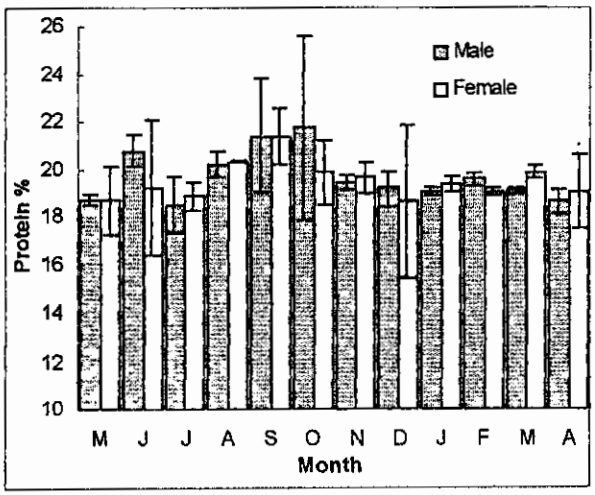

Fig 15: Monthly variation of protein content of body flesh of *Clarias batrachus*. Error bar indicates SD.

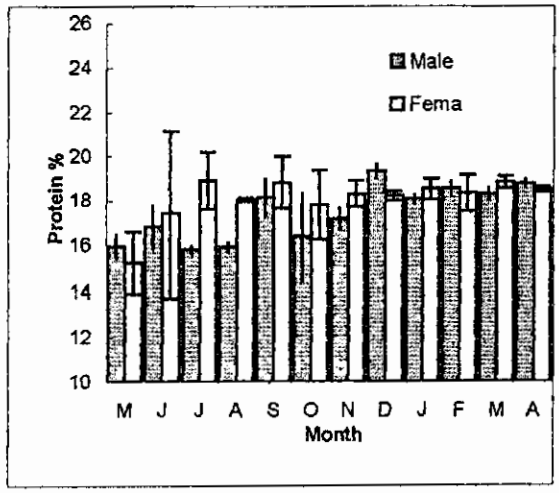

Fig 16: Monthly variation of protein content of whole body of *Clarias batrachus*. Error bar indicates SD.

Different body part: The maximum protein content (19.17%) was found in the middle of male and the minimum (18.35%) was found in the head of male (Table-8).

Table 8: Protein content of different body parts of *Clarias batrachus.*

Body Part	Female (%)±SD	Male(%)±SD
Head	18.73±0.156	18.35±0.214
Middle	18.85±0.064	19.17±0.303
Tail	19.03±0.131	18.59±0.058

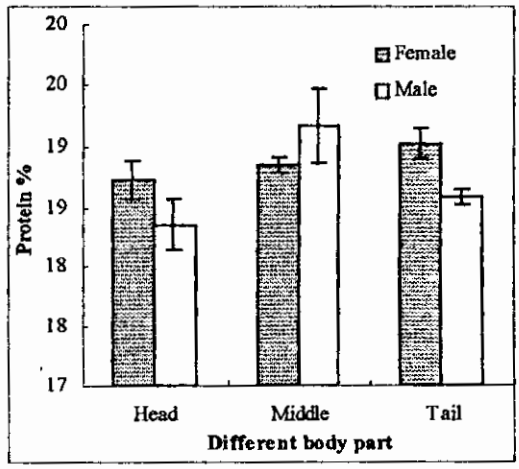

Fig 17: Protein content of different body parts of *Clarias batrachus.* Error bar indicates SD.

3.4.3 Lipid:

Monthly lipid content (as percent) was observed in body flesh and whole body of male and female of *Clarias batrachus* is summarized in the Table: 9.

Body flesh: In male the highest (5.04%) and lowest (1.47%) lipid content were observed in December and May respectively. The maximum lipid content (5.67%) of female was found in January and the minimum (1.34%) was found in the month May. The average lipid content of body flesh of male and female was 2.77% and 2.64% respectively.

Whole body: The maximum (6.87%) and the minimum (0.59%) lipid content of male were found in May and August. For female, the maximum (5.10%) and minimum (0.87%) lipid percent were observed in May and July. The average lipid content of whole body of male and female was 3.34% and 3.48% respectively.

Table 9: Monthly average lipid content of Clarias batrachus

	Male		Female	
Month	Body flesh	Whole fish	Body flesh	Whole fish
May	1.469 ±0.061	6.869 ±0.187	1.338 ±0.152	5.1005 ±0.028
June	2.406 ±0.735	3.001 ±0.147	2.4685 ±0.232	2.9665 ±0.077
July	1.709 ±0.052	0.821 ±0.192	2.2515 ±0.641	0.8705 ±0.124
August	1.53 ±0.244	0.591 ±0.184	2.0405 ±0.620	1.153 ±0.113
September	2.28 ±0.145	2.8 ±0.106	2.4955 ±0.066	2.4785 ±0.141
October	3.601 ±1.214	7.761 ±0.660	2.6115 ±1.131	2.229 ±0.109
November	2.714 ±0.280	2.742 ±0.126	1.9075 ±0.300	2.7975 ±0.303
December	5.038 ±1.314	3.427 ±0.133	4.562 ±1.146	4.9565 ±0.078
January	4.622 ±0.147	4.1515 ±0.240	5.6715 ±0.064	4.8865 ±0.049
February	3.802 ±0.265	4.5385 ±0.320	3.9045 ±0.154	4.967 ±0.048
March	2.574 ±0.091	4.148 ±0.188	2.982 ±0.049	4.5735 ±0.059
April	1.519 ±0.652	3.219 ±0.303	2.476 ±1.352	4.7865 ±0.049
Mean	2.77 ±0.430	3.34 ±0.232	2.64 ±0.490	3.48 ±0.098

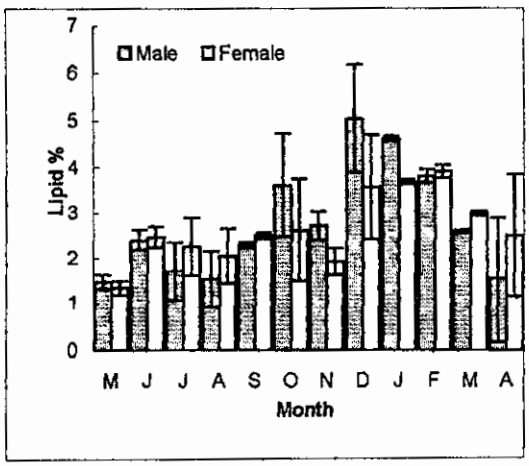

Fig 18: Monthly variation of lipid content of body flesh of *Clarias batrachus*. Error bar indicates SD

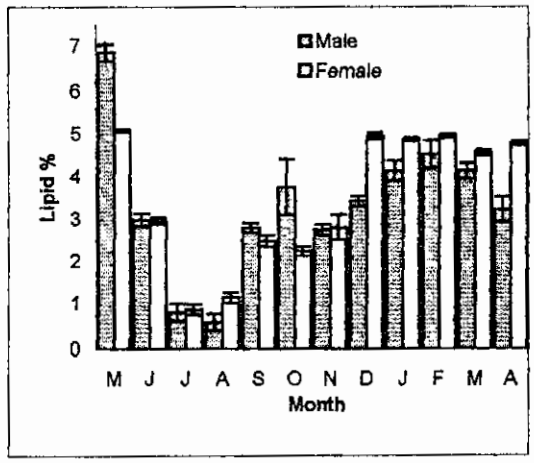

Fig 19: Monthly variation of lipid content of whole body of *Clarias batrachus*. Error bar indicates SD

Different body part: The maximum (1.19%) and minimum (0.57%) lipid content were observed in the middle of male and in the tail of female respectively (Table-10).

Table 10: Lipid content of different body parts of *Clarias batrachus.*

Body Part	Female (%)±SD	Male (%)±SD
Head	0.79 ± 0.019	1.06 ± 0.100
Middle	1.10 ± 0.213	1.19 ± 0.270
Tail	0.57 ± 0.110	1.07 ± 0.066

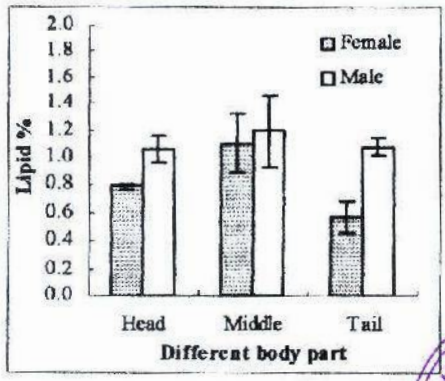

Fig 20: Lipid content of different body parts of *Clarias batrachus.* Error bar indicates SD

3.4.4 Ash:

Monthly ash content (as percent) was observed in body flesh and whole body of male and female of *Clarias batrachus* is shown in the Table:11

Body flesh: The highest ash content (2.05%) of male was found in October and the lowest value (1.17%) was observed in February. For female, the highest (1.82%) and the lowest (1.17%) ash content were found in September and December respectively. The average ash content of body flesh of male and female was 1.48% and 1.41% respectively.

Whole body: In male the maximum (6.96%) and minimum (3.67%) ash value were observed in the month August and October accordingly. For female the maximum ash content (5.435%) was found in June and the minimal value (3.32%) was found in September. The average ash content of body flesh of male and female was 4.85% and 4.63% respectively.

Table 11: Monthly average ash content of Clarias batrachus

	Male		Female	
Month	**Body flesh**	**Whole fish**	**Body flesh**	**Whole fish**
May	1.494 ± 0.015	4.3175 ± 0.009	1.5255 ± 0.099	4.7975 ± 0.032
June	1.346 ± 0.091	5.094 ± 0.168	1.2135 ± 0.215	5.4305 ± 0.037
July	1.466 ± 0.170	5.4305 ± 0.037	1.2645 ± 0.147	5.4055 ± 0.022
August	1.591 ± 0.408	6.961 ± 1.189	1.454 ± 0.343	5.389 ± 0.004
September	1.764 ± 0.166	3.8335 ± 0.295	1.8245 ± 0.154	3.3165 ± 0.151
October	2.053 ± 0.632	3.673 ± 1.131	1.7765 ± 0.011	4.5095 ± 2.119
November	1.708 ± 0.033	5.217 ± 0.137	1.478 ± 0.228	4.422 ± 0.156
December	1.314 ± 0.042	5.6135 ± 0.532	1.173 ± 0.049	3.5985 ± 0.558
January	1.284 ± 0.042	4.822 ± 0.156	1.2235 ± 0.014	4.533 ± 0.403
February	1.165 ± 0.060	4.478 ± 0.222	1.337 ± 0.001	4.1715 ± 0.226
March	1.269 ± 0.222	4.4965 ± 0.163	1.2755 ± 0.065	4.955 ± 0.246
April	1.285 ± 0.024	4.301 ± 0.021	1.361 ± 0.076	5.024 ± 0.267
Mean	1.48 ± 0.148	4.85 ± 0.339	1.41 ± 0.113	4.63 ± 0.356

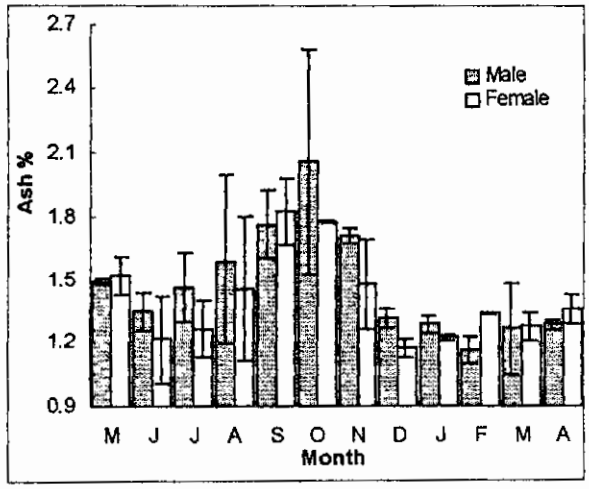

Fig 21: Monthly variation of ash content of body flesh of *Clarias batrachus.* Error bar indicates SD

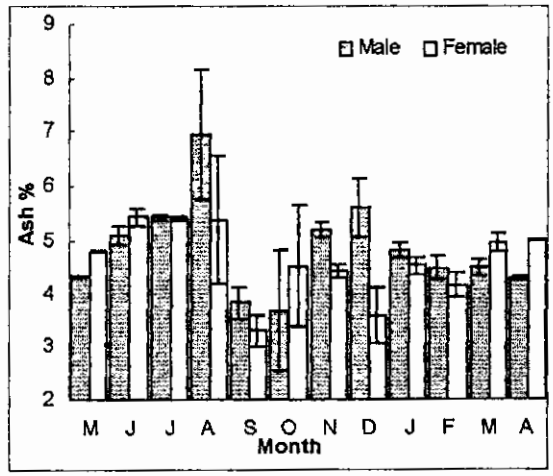

Fig 22: Monthly variation of ash content of whole body of *Clarias batrachus.* Error bar indicates SD

Different body part: The maximum (12.88%) ash content was found in the head of male as well as the minimum (1.66%) was found in the middle of female (Table-12).

Table 12: Ash content of different body parts of *Clarias batrachus.*

Body Part	Female (%)±SD	Male (%)±SD
Head	12.20±0.046	12.88±0.177
Middle	1.66±0.221	2.40±0.039
Tail	2.38±0.223	2.61±0.122

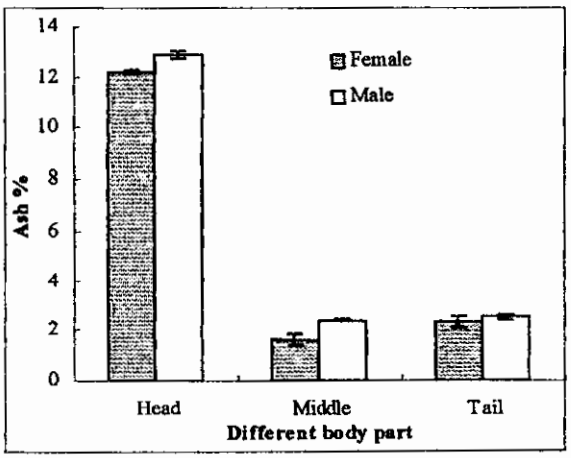

Fig 23: Ash content of different body parts of *Clarias batrachus.* Error bar indicates SD

For male the maximum moisture content (79.31%) was found in the middle portion of the body and the minimum (69.87%) was found in head. Table 6 shows that there is a slight variation of moisture content between middle and tail portion but the moisture content of head is very much lower than the middle and tail portion of the body for both male and female. This may be due to the small amount of meat but large amount of bone in the head.

3.5.2 Protein:

Percentage of protein content in the body flesh of male was highest in the month of October (21.75%) and lowest in the month of July (18.45%). The highest protein content in body flesh of female was found in the month of September (21.35%) and the lowest percentage was found in the month of December (18.62%). The average protein content of male and female was 19.67% and 19.45% respectively. In case of body flesh the protein contents were more or less steady throughout the year except few months. The maximum protein content in whole fish of male was found in the month of December (19.32%) and minimum in the month of July (15.82%). In case of female the maximum protein content and minimum protein content was observed in the month of July (18.89%) and May (15.25%) respectively. The average values of protein content for male and female were 17.44% and 18.08% accordingly. Except few months, there is a slight variation in protein content for whole fish. From the above result it is clear that the protein content in the body flesh is higher than that of whole body. This variation may be due to the presence of bone, fin etc. in the whole body that contain less protein. But there is a minute variation in protein content between two sexes. Monthly variation of protein content that was observed in the study may be due to the consumption of different diet. The whole body contain meat, tail and viscera that are the main cause for composition variation. Chowdhury (1981) mentioned that the protein content of *Clarias batrachus* was 15.84% that is lesser than the result of the present observation. Puwastien *et al.*, worked on *Clarias macrocephalus* and showed that the protein content of that fish was 17%.

In case of body part, the protein content of male was highest (19.17%) in the middle portion of the body and the lowest (18.35%) was in the head. The highest (19.03%) and lowest (18.73%) protein content of female were found in tail and head respectively. Table

8 shows that there is a little variation of protein content among different body parts. This may be due to the size and sex of the fish.

3.5.3 Lipid:

The percentage of lipid content in body flesh of male was maximum in the month of December (5.04%) and minimum in the month of May (1.47%%). The highest and lowest lipid content of body flesh of female were in the month of January (5.67%) and May (1.34%) accordingly. The average lipid content in body flesh of male and female were 2.77% and 2.64% respectively. Table 9 shows that during spawning season (May-June) lipid content is less but the value increases gradually after spawning period and the lipid content is highest in the month of December-January. The maximum lipid content in whole fish of male was in the month of May (6.87%) and the minimum was in the month of August (0.59%). For female the maximum and minimum lipid content were in the month of May (5.10%) and July (0.87%) respectively. The average lipid content in whole body of male and female was found 3.34% and 3.48%. Table 9 shows that after spawning (June-August) lipid content in whole fish is minimum and increases gradually until spawning. The experiment shows that there is a inverse relationship between moisture content and lipid content of the fish. Lipid percentage in whole fish is higher than the percentage of body flesh. This may be due to the reason that fat is stored in the body cavity but absent in the body flesh. Chowdhury (1981) reported that the lipid content of the same species was 2.41% but he did not mention season or the specific part of the body. Puwastein (1999) stated that lipid content of *Clarias macrocephalus* was 8.9%.

In case of different body parts, the maximum (1.19%) and minimum (1.06%) lipid content of male were found in the middle and head respectively. For female the maximum lipid content (1.10%) was found in the middle portion of the body and the minimum lipid content (0.57%) was observed in tail (Table 10). From Table 9 and 10 it is found that the mean value of 12 months of lipid content is higher than those values of different body parts. The lipid content in different body parts was calculated in the month of August at that time the lipid content was very lower than other month of the year.

3.5.4 Ash:

The highest and lowest ash content in body flesh of male was found in the month of October (2.05%) and February (1.17%) respectively. In female fish the highest ash content was observed in September (1.82%) and lowest in December (1.17%). The average ash content in body flesh of male and female were 1.48% and 1.41% respectively. Table 11 shows that there is a little seasonal variation of ash content in body flesh of male and female except September and October. The maximum and minimum ash content in whole fish of male was observed in August (6.96%) and October (3.67%) accordingly. For female the maximum value of ash content in whole body was in the month of July (5.41%) and the minimum was in the month of September (3.32%). The average ash content in whole body of male and female was 4.85% and 4.63% respectively. Slight variation of ash content in whole fish was observed round the year except August. Minute variation of ash content between sexes was also observed. There is large variation of ash content between body flesh and whole fish. This may be due to the presence of fin, bone etc. in the whole fish that contains more ash. For this reason ash content was higher in whole body than body flesh.

In case of different body parts, the maximum (12.88%) and minimum (2.40%) ash content of male were observed in head and middle portion of the body respectively. For female the maximum (12.20%) ash content was found in head and the minimum (2.38%) was found in tail. For both sexes the ash content of middle and tail portion of the body were more or less steady but the ash content in head portion was very much higher than those of middle and tail. This may be occurred due to the reason that the head contains more bone and small amount of meat.

Chapter 4

Conclusion

Conclusion:

The main objective was to study some aspects of biology (sex ratio, fecundity and GSI) and the biochemical composition (protein, lipid, ash and moisture) of *Clarias batrachus*. It is clear from the result of the present study and the discussion made so far that *Clarias batrachus* could be indispensable in diet in respect of their nutritive quality. The sex ratio was not equal to theoretical ratio (1: 1). This could due to over fishing or in certain case, the species sometimes converted their sex as it was observed in other finfish species. So, over fishing and undersize harvesting should be restricted and also fishing in breeding season must be maintained strictly. Once this fish was available in the nature, however, changes in the environment and other factors are threatening for their existence. To ensure the continuity of the biodiversity and retain the species for future generation, proper management and culture techniques of this species should be encouraged.

It was found that considerable variation in the content of the chemical constituents existed within the same species on the fresh weight basis and that the variation was a function of their size. It is well recognized that uncontrolled variables such as the geographical location of fish, seasons, sex, feeding habits and reproductive status affect the nutrient composition of fish. It has been observed that small size species had higher amount of moisture and in most cases large size species had higher value of crude protein and fat. From biochemical composition of *Clarias batrachus*, it is evident that the nutrient content of this species is more or less equal compared to other small valued species even large fish species.

The present study is a preliminary work even conducted in Bangladesh. The major limitation of the present study was various size of sample during the study period. Moreover, the harvesting site of experimental samples was unknown. Due to the collection of fish from local markets there may have some errors in the present study.

This investigation, however, will provide basic information on the biology and biochemical composition of *Clarias batrachus*. The data can also be used as a guideline for promoting fish consumption and further detailed studies.

Chapter 5

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