Some Aspects of Biology and Biochemical Composition of *Channa punctatus* (Bloch, 1794)

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Some Aspects of Biology and Biochemical Composition of *Channa punctatus* (Bloch, 1794)

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DECLARATION

The results submitted in this thesis are entirely of the candidate's own investigations 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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DEDICATED TO

My Beloved Parents

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ABSTRACT

To study the biology of the snakehead, *Channa punctatus* 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: 0.57. Fecundity of the fish ranged from 2274 to 9795 in fishes total length ranged of 18-15.5 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 ($P<>0.05$). 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 June with a peak in June for male and between May to June with a peak in May for female. Furthermore, GSI revealed that *C punctatus* has a short spawning season.

The effects of the parts of body, size class, sun drying, oven drying, boiling, frying, freezing and eggs on proximate composition of *C punctatus* were determined. Large size fishes were high in protein (19.37 ± 0.21%) and fat (1.10 ± 0.03%) and low in ash (1.75 ± 0.02%) and moisture (79.64 ± 0.10%) whilst small size fish low in protein (17.77 ± 0.14%) and fat (0.73 ± 0.14%) and high in moisture (82.73 ±1.03%). For different body parts, the protein content was higher in the head portion of male (19.86 ± 0.02%) than female; the lipid content was higher in different parts of male than female. The percentage of ash content ranged between 1.25 and 3.54 % and among the different portions of male and female fish, moisture content was the highest in tail (77.43 ± 0.89%) and the lowest in head (76.31 ± 0.09%) of male. These were inversely related to female.

The protein, lipid and ash content in the fish flesh, sun dried fish, oven dried fish, boiled fish, fried fish, freezed fish and fish eggs ranged from 18.63 ± 1.29 to 66.51 ± 4.87, 0.56 ± 0.45 to 30.03 ± 1.40 and 1.20 ± 0.14 to 34.17 ± 1.29%, respectively. In general dried fish and fish eggs showed higher protein and lipid content.

Monthly changes in nutrient content like protein, lipid, ash and moisture were assessed in the snakehead, *C punctatus* in both wet weight (body flesh) and dry weight (whole body) basis from the month May 2002 to April 2003. The highest value of protein in the body flesh was found in November (22.34 ± 0.31%) for Male and in September (22.57 ± 2.02%) for

female. The highest value of lipid was found in February for male (2.49 ± 0.17%) and female (2.64 ± 0.32%). The highest value of ash was found in September (2.12 ± 057%) for male and February (2.28 ± 1.04%) for female. The highest value of moisture content was found in August for male (81.36 ± 1.36%) and female (80.01 ± 0.14%). On the dry weight (whole body) basis, the highest value of protein (78.67 ± 0.35%), lipid (21.60 ± 0%), ash (34.17 ± 1.29%) and moisture (75.48%) was found in January, April, July and November, respectively for male. On the other hand, the highest value of protein (76.13 ± 0.83%), lipid (19.58 ± 0.12%), ash (28.59 ± 0.49%) and moisture (76.53%) was found in September, March, June and October, respectively for female fish. From the nutritional point of view it shows that the nutrient content of snakehead fish *C punctatus* is undeniable compared to other small and large fishes.

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Biology of *Channa punctatus* (Sex ratio, fecundity & GSI)

1.0 Introduction

Bangladesh steps in a new millennium with high hopes and aspirations, strengths and weakness and new opportunities and constraints for its development. Bangladesh is blessed with vast natural resources and fisheries resources are more significant among these. Fisheries sector plays a vital role from the point of nutrition supply, employment generation, foreign exchange earning and other areas of economy.

Prior to 1970 many different small indigenous fishes like Koi, Taki, Veda, Pábda, Gulsha, Mola, Puti, Shing, and Magur etc were abundant in almost all the fresh water areas of Bangladesh. Due to the price and availability, these species of fish always occupy an important part of the daily consumption of all classes of people especially low-income groups. Moreover, these small indigenous fish were delicious and popular food items in their diet. Only about two decades ago murrels were available in every water bodies in Bangladesh. But recently, due to various natural degradation and man made structure, these fish species are threatening for their existence.

Bangladesh is comprised vast water area which includes 5308657 ha of fresh water with an excellent propagation of fish. Total fish production in our country during the year 2000 – 2001 was about 1781,057 MT, which 1401,560 MT of were produced from fresh water fish. Snakehead fish production was about 56441 MT and contribution of snakehead fish was about 3.17% to the total production of fish (DOF, 2002).

Bangladesh is one of the populous countries in the world. Here 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 increasing of population of Bangladesh. About 75% people have been suffering from malnutrition. So in the 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. Hossain *et al.* (1994) mentioned that among the fishing communities' small fish occupy an important position as a popular food item. Small indigenous fishes have a high nutritional value in terms of both protein and presence of micronutrients, vitamins, and minerals (Akhteruzzaman *et al.*, 1997). They contain large amount of calcium and most likely also iron and zinc (Tripathi, 1997). But over the last several decades the supply of fish had been declining and fish was becoming scarce day-by-day. As a result daily per capita consumption of fish has dropped. Per capita annual fish needed in our country about 18 kg but per capita annual fish intake is about 12 kg (DOF, 2002). Unfortunately, the present productions are unable to fulfill the country's demand and as a result there is a severe chronic deficiency in essential nutrients in the diet of the people of Bangladesh (Ahmed *et al.*, 1977).

There are 260 freshwater species in Bangladesh (DOF, 2002). *C punctatus* is an important species of the class Osteichthyes under the phylum Chordata. The group name of this species is snakehead. They are belonging to the family Channidae. Snakehead is also known as murrels and serpent-headed fish in different regions of the world. Locally *C punctatus* is called 'taki, lata, lati, okoland chaitan' (Rahman, 1989). This species is available in smaller water bodies like ditches, ponds, lakes, beels, haors, baors, swamps and ephemeral water bodies of the inland water (Rahman, 1989). They can easily be grown in the neglected fallow water areas and margins of agricultural lands, roads and houses. Every one can also capture the small fishes with their hands and simple gears from such water bodies where large fishes cannot survive (Hossain *et al.*, 1994). The more common murrel species found in Bangladesh are *Channa punctatus, C. striatus, C. marulius, C. gachua and C. orientalis.* So far, there is no systematic investigations have been reported on fecundity, sex ratio and GSI of *C punctatus* except the taxonomic observation done by Rahman (1989). Though the study of the biology of *C punctatus* comprises many aspects, here a comprehensive account of its sex ratio, fecundity and gonadosomatic index (GSI) have been presented.

A knowledge of sex ratio of catches is to help in understanding whether any differential fishing exist, to know the appropriate number of male and female of fishes can be mated during artificial spawning or in natural spawning grounds and also to devise means of ensuing proportional fishing of two sexes (Alam, 1994). Overall the knowledge of sex ratio of fish population is essential for the management of fishery.

The reproductive capacity of a population is a function of the fecundity of the females. Fecundity is the general term used to describe the number of mature eggs produced by an individual female (Lagler, 1949). Fecundity or reproductive potential can be defined as the

= 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.

Huda *et al.* (1997) reported that spawning occurred twice a year i.e. February to April and September to October, as evidenced by ova diameter polygons, GSI and Kn values. The equations between fecundity and total length, fish weight, ovary weight and ovary length have been determined. Mean fecundity was 38318. Male-female sex ratio was found to be 1.6: 1.

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

Sarker *et al.* (2002) showed the fecundity and gonado somatic index *of Mystus gulio*. At different length ranged from 10-20 cm of fish showed different fecundity and 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 16-18 g. 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 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 in rainbow w trout

Doha and Hye (1970) reported that the fecundity of *Hilsa ilisha* was found to be vary high ranging from 348318 (fork length of fish 273 mm.) to 1465969 (fork length of fish 42 mm) in number. A regression equation, Y= 1000 (-1679.282454 + 7.06586x), with r = 0.916. It appears that the fork length and the fecundity bear a linear relationship.

Shafi and Quddus (1974) reported that the fecundity of the common puti, *Puntius stigma* from a tributary of the river Buriganga was estimated by the gravimetric method.

Mathematical relationships of fecundity with length, weight and gonad weight and of gonad weight with length and weight have been estimated.

Dewan and Doha (1979) reported that the fecundity of seven species of pond fishes viz. *Chela phulo, Esomus danrica, Amblypharyndodon mola, Aplocheilus panchax, Ambassis nama, Glossogobius giuris and Colisa lalius* ranging from 176–3930, 392-2412, 1021-13815, 39-486, 110-2448, 376-24668 and 127-2309, respectively.

Mustafa *et al.* (1980) studied the food, feeding habits and fecundity of the fresh water perch, *Nandus nandus.* They reported that fish length ranged from 6 to 16.8 cm and body weight ranged from 5 to 60 g. The fecundity ranged from 7381 eggs for a fish with body length of 9.7 cm and 46222 eggs for a fish with body length of 13.5 cm. The fish was highly fecund for its size. Breeding season ranged from June to July. Mathematical relationship between body length– fecundity and body weight- fecundity were found to be linear.

Bhuiyan and Rahman (1983) reported that the fecundity of *Channa gachua* (Hamilton) ranged from 487 to 4482 eggs. The r-values for F/TL, F/TW, F/GL, and F/GW, were 0.757743, 0.760288, 0.895879 and 0.932916, respectively.

Mustafa *et al.* (1983) studied the biology of *Puntius sarana* and they reported that its fecundity ranged from 18925 to 78929 and length between 155 mm and 250 mm. Mathematical relationship between fecundity and total length, fecundity and body weight, and fecundity and ovary weight were linear where as a curvilinear relationship was noted between fecundity and standard length of the fish.

Banu *et al.* (1985) reported the fecundity of *Anabus testudineus* (Bloch) varied from 12355 eggs (a fish with total length 12.3 cm and weight 30.24 gm) to 41820 eggs (a fish with total length 15.5 cm and weight 66.62 gm). The correlation co-efficient was highest in case of relationship between fecundity and ovary weight, which indicated that fecundity bears strong relationship with ovary weight. The fecundity was found to bear linear relationship with total length, somatic and ovary weight.

Danwattana and Nakorn (1985) reported that the number of eggs of *Channa striatus* was within the rang of 12970-60283 and having size of 250-850 g in weight and 29.5-45.8 cm in length.

Barua *et al* (1986) are of opinion that *Clarias batrachus* has a single spawning season that last from May to July. Gonado Somatic Index varies in both sexes, reaching the peak in the month of June. The also observed that the mean GSI value was the highest during the highest temperature and the highest rain fall of the year.

Azadi *et al* (1987) reported that fecundity of the *Mystus vittatus* (Bloch) with the length between 89 and 115 mm was found to range from 2515-9789. 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 *Heteropneustus fossilis* ranged from 1200-23500, total length from 103 mm- 298 mm and body weight 7.5g to 73 g 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.

3.0 Materials and Methods

For the present study, a total of 176 random brood specimens of *Channa punctatus* were collected monthly from May 2002 to April 2003 from the local market, Gallamary Bazar, Khulna. They were firstly cleaned by blotting paper and 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 electronic balance (B-303S). The length and weight was recorded in the nearest centimeter and gram respectively. The experiment was conducted in the Laboratory of Fisheries and Marine Resources Technology Discipline, Khulna University, Khulna.

3.1 Sex ratio:

The fishes were dissected and the ovary and testes were removed very carefully. In the present studies sex of every young fishes was ascertained by examination of gonad under microscope. The ovaries were identified by the presence of round 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 sex ratio for the difference, if any, from the hypothetical ratio 1: 1 (Hoda, 1997).

3.2 Fecundity:

For the fecundity study, 21 female species were randomly collected from March 2003 to October 2002. The fishes were dissected and the ovary was removed very carefully. In the present studies sex of every young fishes was ascertained by examination of gonad under microscope. The ovaries were identified by the presence of rounded ova in them. The varies were preserved in 5% formalin with proper labeling to permit hardening of the ova to facilitate for subsequent studies.

ascertained by examination of gonad under microscope. The ovaries were identified by the presence of round ova in them and testes by the absence of any round ova in them. The weight of each ovary and testes were taken with the help of electronic balance (B-303S).

GSI was calculated according to the following equation cited by Parameswarn *et al* (1974). It can be calculated to ascertain the spawning season of a fish and the seasonal analysis would indicate the peak spawning activity. 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).

4.0 Results

4.1 Sex Ratio:

The number of the two sexes was recorded by observing the gonad through dissection. During the one-year study period (May-02 to April-03) out of 176 specimens, 112 (63.6%) were males and 64 (36.4%) were females (Table-01). It was seen that the two sexes were not present in the same proportion through out the year (Fig-01). The percentages of males were comparatively higher in the month of May (83.3%) and November (72.2%) while the females were higher in the month of September (54.5%) and April (53.3%).

Taking the complete sample as whole, the overall male-female sex ratio was 1: 0.57 which is highly significantly different from the theoretically expected ratio 1: 1. The present ratio of males to females for the entire period of investigations indicated predominance of males over females. The chi-square value for month wise sample showed that the sex ratio of combined sex was insignificant. The calculated value of $\chi 2$ was 0.6834782 which was insignificant at 5% level of significance with 11 degrees of freedom (Appendix-1).

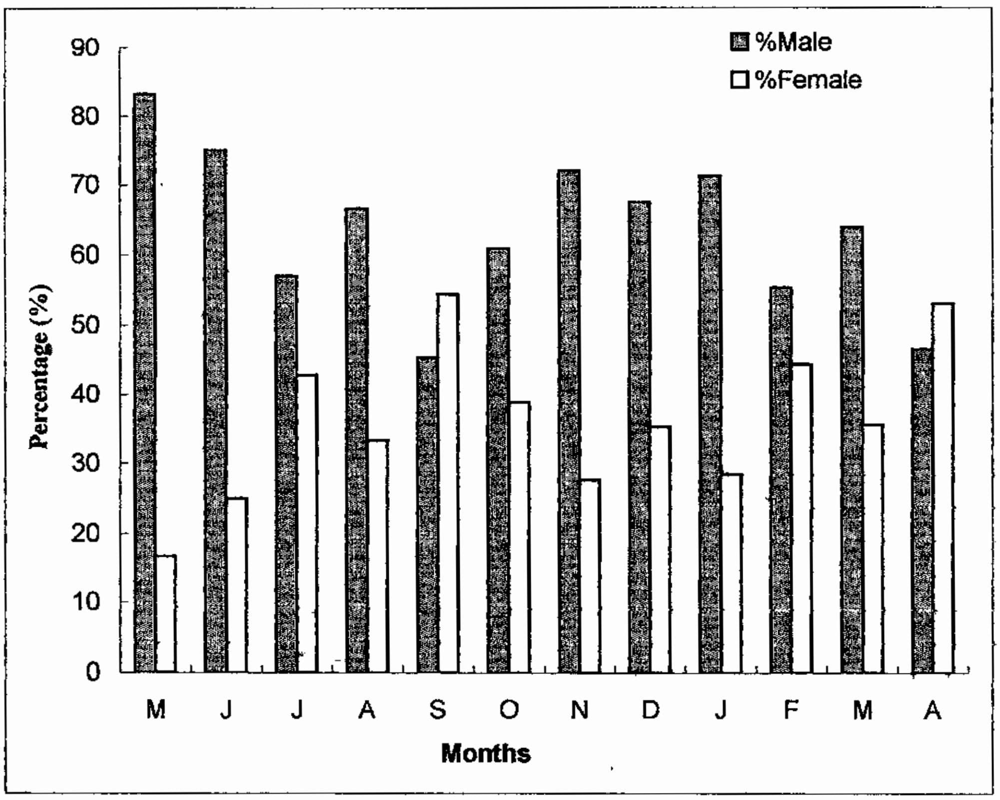


Fig-01: Sex ratio of *Channa punctatus.*

4.2 Fecundity:

Breeding season was indicated by the presence of berried females in the catch. Accordingly, 21 berried females were randomly collected and analyzed for fecundity during eight months. Eggs were not available from November to February. Fecundity, defined as the number of eggs produced by an individual female (Lagler, 1949) varied in *Channa punctatus* from minimum 2274 to maximum 9795 eggs in fishes having a total length 18 cm (64.524 g) and a total length 15.5 cm (36.514 g), respectively (Table-02). The mean numbers of eggs were 4878 of a fish with a mean total length 17.6 ± 1.347 cm and a mean body weight of 56.073 ± 14.563g (Table-02). From the Table-02 it is reveled that the peak breading season is in May.

Total Length-Fecundity Relationship

The scatter diagram revealed the presence of a linear relationship between total lengths and fecundity. The regression line of fecundity on total length was found to be linear and slightly positive (Fig-02). So the relationship can be expressed as: Y= 0.3746x + 3.1878 (r = 0.071, n = 21, t_{cal}= 62.3717)

The co-efficient of correlation (r = 0.071) was tested by t-test and found significant at 5% level of significance ($t_{0.05}$=2.085) with 21 d.f (Appendix-2).

Standard Length- Fecundity Relationship

The scatter diagram revealed the presence of a linear relationship between standard lengths and fecundity. The regression line of fecundity on standard length was found to be linear and slightly positive (Fig-03). So the relationship can be expressed as: Y= 0.5717x + 2.9888 (r = 0.11, n = 21, t_{cal} = 64.934)

The co-efficient of correlation (r = 0.11) was tested by t-test and found significant at 5% level of significance ($t_{0.05}$=2.085) with 21 d.f (Appendix-2).

Total body Weight- Fecundity Relationship

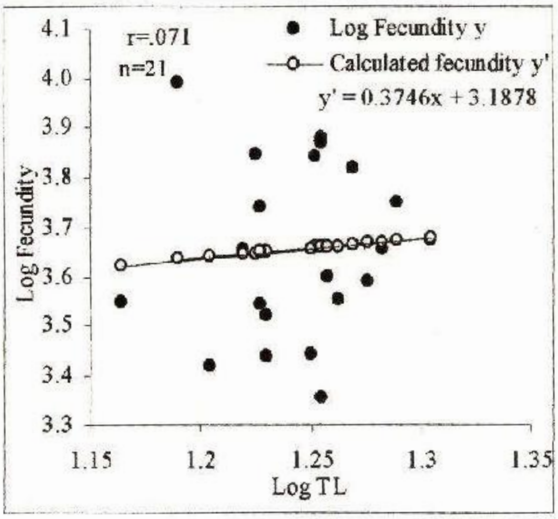
The scatter diagram revealed the presence of a linear relationship between total body weight and fecundity. The regression line of fecundity on total body weight was found to be linear and slightly negative (Fig-04). So the relationship can be expressed as: $Y = -0.1425x + 3.9013$ ($r = 0.091$, $n = 21$, $t_{cal} = 40.3463$

The co-efficient of correlation ($r = 0.091$) was tested by t-test and found significant at 5% level of significance ($t_{0.05}=2.085$) with 21 d.f (Appendix-2).

Gonad Weight- Fecundity Relationship

The scatter diagram revealed the presence of a linear relationship between gonad weights and fecundity. The regression line of fecundity on gonad weight was found to be linear and positive (Fig-05). So the relationship can be expressed as: $Y = 0.4654x + 3.512$ ($r = 0.524$, $n = 21$, $t_{cal} = 83.4875$)

The co-efficient of correlation ($r = 0.524$) was tested by t-test and found significant at 5% level of significance ($t_{0.05}=2.085$) with 21 d.f (Appendix-2).



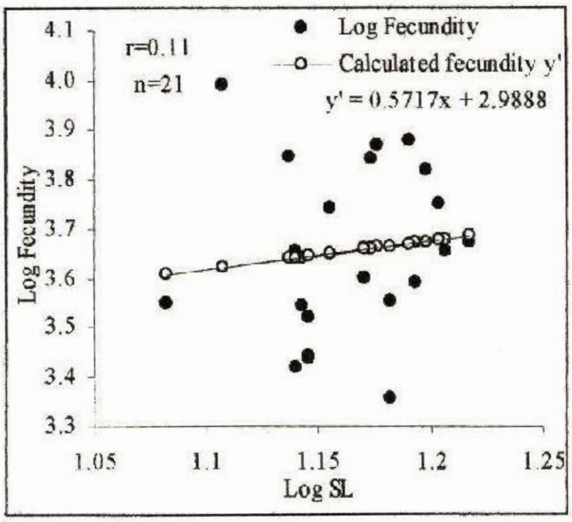
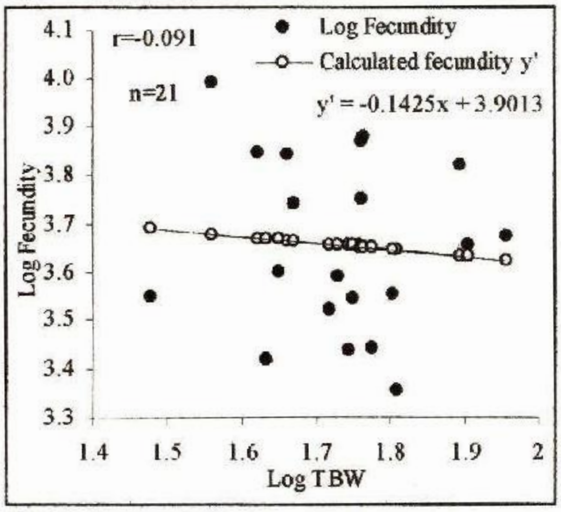


Fig-02: Relationship between Log total length and Log fecundity of *Channa punctatus.*

Fig-03: Relationship between Log standard length and Log fecundity of *Channa punctatus*



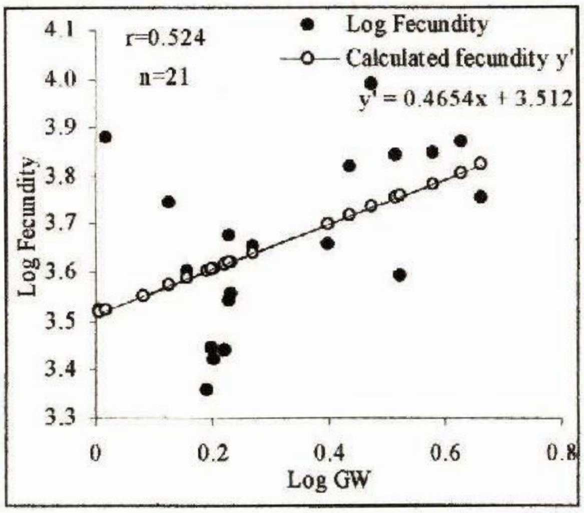


Fig-04: Relationship between Log total body weight and Log fecundity of *Channa punctatus*

Fig-05: Relationship between Log gonad weight and Log fecundity of *Channa punctatus*

4.3 Gonado-Somatic Index:

In the present study, 176 male and female *Channa punctatus* were examined to determine the GSI (Table-03 & Table-04). Calculating the mean GSI for each sex in each sample followed the seasonal cycle of gonad development. The gonado-somatic index, which is indicative of breeding season in fish, has been calculated month wise and to establish spawning season of *Channa punctatus* has been plotted against months.

Male

Monthly average GSI values of males are graphically represented in Fig.-06. GSI values of males ranged from 0.019 ± 0.024 to 0 .0917 ± 0.015. The GSI values generally increased in April and from May to June increased highly with a peak in June (0 .0917 ± 0.015). GSI values began to fall steeply from August.

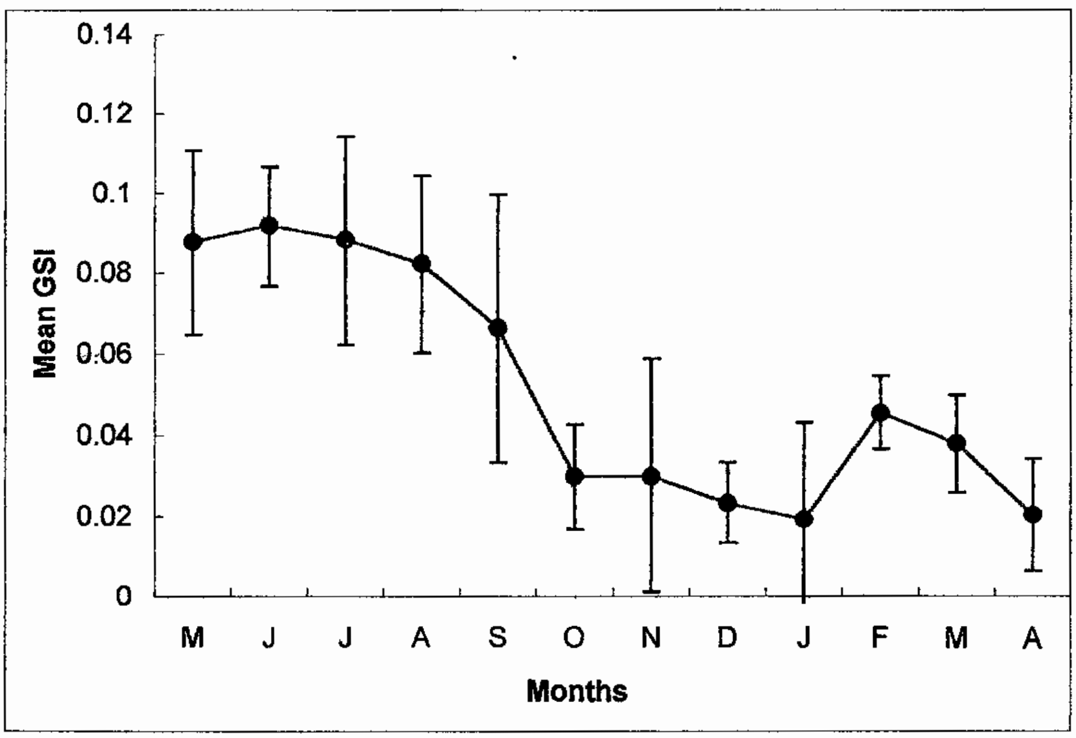


Fig-06: Seasonal variation in gonad weight of male *Channa punctatus*. Error bar indicates standard deviation.

Female

Monthly average GSI values of females are graphically represented in Fig-07. GSI values ranged from 0.2125 ± 0.081 to 7.8705 ± 1.034. The GSI values began to increase in February and from May to June increased sharply with a peak in May (7.8705 ± 1.034). GSI values began to fall steeply from August.

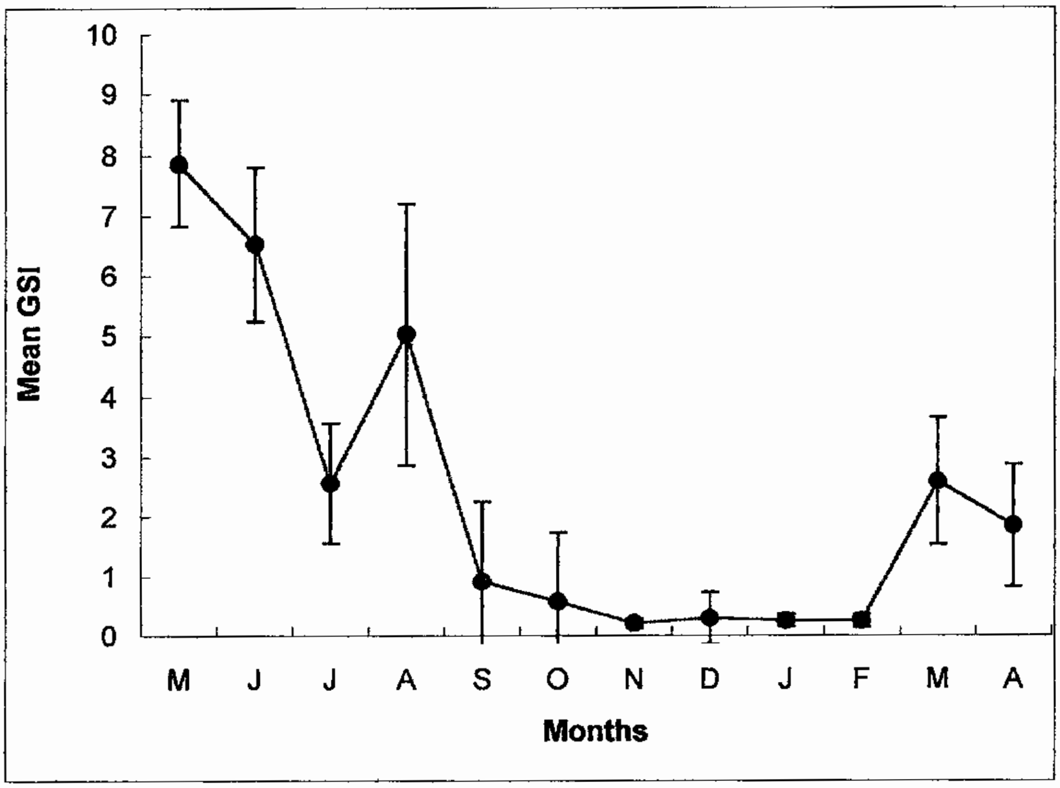


Fig-07: Seasonal variation in gonad weight of female *Channa punctatus*. Error bar indicates standard deviation.

5.0 Discussion

5.1 Sex Ratio:

In the present study for twelve months (May-02 to April-03) the sex ratio (male: female) of the collected samples was 1: 0.57. Slightly disproportionate occurrence of the number of the two sexes of *C punctatus*. Variations from this are often observed in fish may be due to different behavior of sexes, environmental conditions, fishing etc. Bal and Rao (1969) and Dan (1977) have shown the sex ratio of catfish, *Arius tenuispinis* as male: female (1: 0.64) that was almost similar to the present findings. But Jhingran (1961) reported on *Setipinna phasa* with the occurrence of two sexes, males and females in the ratio of 1: 1.92. Monthly analysis of sex ratio showed (Fig-01& Table-01) that the females were dominated over males during the peak period of spawning (April) except September.

5.2 Fecundity:

During eight months observation it is revealed that the fecundity of *Channa punctatus* varied from 2274 (for a fish for total length 18 cm, standard length 15.2 cm, body weight 64.524g and gonad weight 1.553g) to 9795 (for a fish for total length 15.5 cm, standard length 12.8 cm, body weight 36.514g and gonad weight 2.981g). The average number of eggs were found 4878 for a female fish with an average total length, standard length, total body weight and gonad weight of 17.6 ± 1.34 cm, 14.6 ± 1.13 cm, 56.073 ± 14.563 g and 2.238 ± 1.083 g, respectively (Table-02).

The range of fecundity was quite high and maximum fecundity recorded in May clearly indicated it's peak spawning time. The numbers of eggs produced by the fish was dependent on the size of the fish (Thakur, 1978, Shafi and Quddus, 1974 and Rishi and Kaul, 1982). The average fecundity varied among the months except in breeding season. In all the fishes the numbers of eggs increased with the increase in their length (Dewan and Doha, 1979) that agreed with present study. For the difference in the fecundity such factors viz. nutritional state (body weight, body length), time of spawning, maturity stages and racial characteristics may be responsible (Healey, 1971 & Bagenal, 1966).

Fecundity of *C punctatus* varied from 2268 eggs for a female fish with a length group of 121-140 mm and weight of 53.2 g to 4545 eggs for a female fish with a length group of 281-

300 mm and weight of 117.5 g. The average number of eggs was 3360 for a female with an average total length 210.5 mm and average weight of 86.61 g (Latifa, 2001). She also mentioned that the relationship between fecundity- length, fecundity- ovary weight and fecundity- body weight were linear and positive. Present study supported Latifa's findings except fecundity- body weight relationship. In case of total body weight and fecundity Jhingran (1961) was shown the positive relation for *Setipinna phsa*. The present finding merely differed with other relationship of fecundity- body weight and this could be the species variation

In the present investigation, a linear and positive relationship was found between total length and fecundity. Kader *et al.* (1982), Afroza and Hossain (1983) and Millar (1984) reported linear positive relationship between total length and fecundity in various fish species.

Linear positive relationship between gonad weight and fecundity was also reported by the workers like Shafi and Mustafa (1976); Shafi *et al.* (1978 & 1979), De and Mitra (1981), Rahman (1982) and Nargis *et al.* (1983) in different species of fish. Their investigations agree with the result of the present findings.

A comparison of the correlation co-efficient of fecundity-total length ($r = 0.072$), fecundity-standard length ($r = 0.11$), fecundity-body weight ($r = 0.092$) and fecundity-gonad weight ($r = 0.524$) indicated much closer relationship of fecundity with gonad weight than other factors like total length, standard length and body weight. Shafi *et al,* (1978 & 1979), Crivelli (1981), Bhuiyan and Rahman (1982), Nargis *et al.* (1983), and Kader (1984) also reported the relationship between gonad weight and fecundity to be the most significant than that between fecundity and other parameters.

5.3 Gonado-Somatic Index:

Male

The male fish had higher proportion of body weight and testes weight in the month of June that was a good indication of its peak maturity. It was evident from the present study that the GSI values increased slowly from February to August and from May to June increased highly with a peak in June (0.092 ± 0.015). GSI values began to fall steeply from August. Mustafa *et al.* (2000) studied the GSI on the ribbonfish, *Lepturacanthus savala.* His finding was similar to the present result. It is familiar that GSI increases with the maturation of fish, being maximum during the period of peak maturity and declining abruptly thereafter (Parameswar *et al.*, 1974). Pandian (1967) reported spawning of *M. gulio* was found during May to October with a peak in the month of May and June. Hoda *et al.* (1997) observed the male GSI range of *Euryglossa orientalis* from 0.139 to 0.231. The male fish of *Clarias batrachus* had highest GSI values in the month of March (Ali *et al.*, 1992-1994). The present findings observed that the highest GSI value was in the month of June. This insignificant variation in GSI may be due to the variation of habitat or food availability, size, age, spawning period of fish.

Female

The female fish had higher proportion of body weight and ovary weight in the month of May. It is interesting that female matures earlier than the male. It was evident from the present study that the GSI values increased slowly from February to August and from May to June increased highly with peak in May (7.87 ± 1.034). GSI values began to fall steeply from August. Hoda *et al.* (1997) observed the female GSI range of *Euryglossa orientalis* from 0.379 to 3.246 and showed the GSI values began to increase in August and January building up to a maximum September-October and February to March thereafter they rapidly declined, reaching their minimum in April. Sarker *et al.* (2002) observed the highest GSI value of *Mystus gulio* in the month of July. This variation would be due to spawning season differences. Khan *et al.* (2002) observed the GSI of *Plotosus canius* increases steadily from April reaching in peak in July and becomes least in August. The present finding was supported by the observation of Khan *et al.* (2002). The female fish of *Clarias batrachus* had highest GSI in the month of April (Ali *et al.*, 1992-94), which in the present finding, it is in the month of May. This variation could be attributed habited differentiations, food availability or environmental factors.

Tables

Table-01: Sex ratio of *Channa punctatus* in different months

Months	No. of obs	Male	Female	%Male	%Female	M : F
May	18	15	3	83.33	16.66	1: 0.20
June	12	9	3	75	25	1: 0.33
July	7	4	3	57.14	42.85	1: 0.75
August	12	8	4	66.66	33.33	1: 0.50
September	22	10	12	45.45	54.54	1: 1.20
October	18	11	7	61.11	38.88	1: 0.64
November	18	13	5	72.22	27.77	1: 0.38
December	17	11	6	67.70	35.29	1: 0.55
January	14	10	4	71.42	28.57	1: 0.40
February	9	5	4	55.55	44.44	1: 0.80
March	14	9	5	64.28	35.71	1: 0.56
April	15	7	8	46.66	53.33	1: 1.14
Total	176	112	64	63.63	36.36	1: 0.57

Table-02: Fecundity of female *Channa punctatus* with their respective total length, standard length, body weight and gonad weight.

Months	TL (cm)	SL (cm)	TBW (g)	GW (g)	Fecundity	Log TL	Log SL	Log TBW	Log GW	Log Fecundity
March	18	15.2	64.524	1.553	2274	1.255	1.181	1.809	0.191	3.356
	17	14	55.745	1.669	2745	1.230	1.146	1.746	0.222	3.438
	16.6	13.8	57.577	1.87	4485	1.220	1.139	1.760	0.271	3.651
	18.6	15.8	78.722	2.743	6605	1.269	1.198	1.896	0.438	3.819
April	17	14	52.544	1.021	3321	1.230	1.146	1.720	0.009	3.521
	18.3	15.2	63.961	1.71	3580	1.262	1.181	1.805	0.233	3.553
	20.2	16.5	90.581	1.701	4704	1.305	1.217	1.957	0.230	3.672
	16.9	13.9	56.264	1.697	3485	1.227	1.143	1.750	0.229	3.542
	19.2	16.1	80.91	2.507	4513	1.283	1.206	1.908	0.399	3.654
May	18	15	57.988	4.285	7427	1.255	1.176	1.763	0.632	3.870
	16.8	13.7	41.941	3.8	7015	1.225	1.136	1.622	0.579	3.846
	17.9	14.9	45.91	3.288	6925	1.252	1.173	1.661	0.516	3.840
June	19.5	16	58	4.605	5659	1.290	1.204	1.763	0.663	3.752
	18.9	15.6	53.714	3.346	3906	1.276	1.193	1.730	0.524	3.591
July	18	15.5	58.238	1.044	7571	1.255	1.190	1.765	0.018	3.879
	16	13.8	43.097	1.596	2619	1.204	1.139	1.634	0.203	3.418
August	18.1	14.8	44.775	1.446	3970	1.257	1.170	1.651	0.160	3.598
	15.5	12.8	36.514	2.981	9795	1.190	1.107	1.562	0.474	3.991
September	16.9	14.3	46.887	1.349	5531	1.227	1.155	1.671	0.130	3.742
	14.6	12.1	30.062	1.209	3534	1.164	1.082	1.478	0.082	3.548
October	17.8	14	59.574	1.585	2780	1.250	1.146	1.775	0.200	3.444
Mean±SD	17.6 ±1.34	14.6±1.12	56.073±14.563	2.238±1.083	4878					

Table-03: GSI of male *Channa punctatus* in different months. Each value represents Mean ± SD.

Months	TL (cm)	SL (cm)	TBW (g)	GW (g)	GSI	Mean GSI
May	19.1	15.4	60.412	0.059	0.0976627	0.08776 (± 0.023)
	16.6	13.5	38.588	0.044	0.1140251	
	19.3	15.6	68.459	0.067	0.0978688	
	22.3	17.8	74.428	0.078	0.1047993	
	20.3	16.8	99.156	0.03	0.0302554	
	19	15.6	65.003	0.041	0.063074	
	18.1	15	53.611	0.05	0.0932644	
	21.6	17.4	91.561	0.045	0.0491476	
	19.3	15.5	65.189	0.071	0.1089141	
	22.3	17.8	105.876	0.082	0.0774491	
	20.3	16.3	85.103	0.073	0.0857784	
	19	15.5	58.026	0.056	0.0965085	
	15.5	12.6	35.234	0.031	0.0879832	
	18.8	15.2	59.954	0.057	0.0950729	
	18.5	15.1	51.482	0.059	0.1146032	
June	17.8	14.7	52.004	0.042	0.080763	0.091712 (± 0.015)
	19	15.5	57.624	0.05	0.0867694	
	20.4	16.5	66.777	0.067	0.1003339	
	18.4	14.8	53.64	0.039	0.0727069	
	15.5	12.6	35.234	0.031	0.0879832	
	18.4	15	49.004	0.042	0.0857073	
	20.4	16.7	61.917	0.076	0.122745	
	16.8	13.6	40.748	0.043	0.1055267	
	18.5	15	57.92	0.048	0.0828729	
July	22.1	18.3	96.165	0.076	0.0790308	0.088292 (± 0.026)
	17.5	15	54.882	0.035	0.0637732	
	24.5	20.2	126.863	0.107	0.084343	
	20.5	16.5	73.003	0.092	0.1260222	
August	17.8	14.5	53.068	0.055	0.1036406	0.08234 (± 0.022)
	15.6	12.5	38.275	0.025	0.0653168	
	18.9	15	54.801	0.057	0.1040127	
	17.6	14.5	53.502	0.021	0.0392509	
	17.6	13.8	47.621	0.047	0.098696	
	17.4	14	52.214	0.038	0.0727774	
	17.5	14	54.768	0.047	0.0858165	
	17.3	14.9	57.409	0.0512	0.0891846	
September	17.1	14.3	48.316	0.037	0.0765792	0.06648 (± 0.033)
	16.4	13.6	39.794	0.022	0.0552847	
	15.8	13.1	41.702	0.014	0.0335715	
	16.5	14.2	39.837	0.029	0.0727966	
	17.5	14.1	46.478	0.024	0.0516373	
	17.1	13.7	43.006	0.018	0.0418546	
	16.4	13	46.238	0.037	0.0800208	
	21.3	17	82.43	0.023	0.0279025	
	14.6	12.1	30.062	0.044	0.1463642	
	17.8	15.1	55.844	0.044	0.0787909	

October	16	13.3	41.301	0.008	0.01937	0.03002 (± 0.013)
	16.3	13.3	33.531	0.013	0.0387701	
	19.5	16.5	59.53	0.017	0.028557	
	17.8	15	59.574	0.004	0.0067143	
	15	13	34.802	0.008	0.0229872	
	19.4	15.6	48.585	0.029	0.0596892	
	16.4	13.5	43.175	0.018	0.0416908	
	20.8	16.4	65.458	0.018	0.0274985	
	18.8	15.5	70.746	0.021	0.0296837	
	18.3	14.7	55.91	0.015	0.0268288	
	17.3	14.4	45.669	0.013	0.0284657	
November	17	14.3	47.85	0.017	0.0355277	0.03013 (± 0.029)
	17.8	15	48.671	0.007	0.0143823	
	16.3	13.6	49.815	0.022	0.0441634	
	16.6	14	54.549	0.019	0.0348311	
	16.4	13.6	47.819	0.015	0.0313683	
	15.4	12.7	41.724	0.009	0.0215703	
	17	14.1	56	0.012	0.0214286	
	17.3	14.4	48.411	0.005	0.0103282	
	18.6	15.4	54.29	0.007	0.0128937	
	17.5	14.5	59.504	0.005	0.0084028	
	16.1	13.8	39.422	0.009	0.0228299	
	16.2	13.4	48.955	0.059	0.1205188	
	17.4	14.5	52.2	0.007	0.01341	
December	19.8	16.5	79.12	0.034	0.0429727	0.02358 (± 0.01)
	20.1	17	80.888	0.016	0.0197804	
	17.5	14.5	53.532	0.008	0.0149443	
	16.4	13.5	43.478	0.006	0.0138001	
	19.4	16.2	57.433	0.014	0.0243762	
	17.5	14.4	58.131	0.015	0.0258038	
	18.9	15.6	67.737	0.006	0.0088578	
	16.7	14	48.04	0.011	0.0228976	
	19	15.8	67.773	0.027	0.0398389	
	14.6	12	31.815	0.009	0.0282885	
	16.3	13.5	39.162	0.007	0.0178745	
January	17.1	14.2	52.775	0.006	0.011369	0.0195 (± 0.024)
	18.1	15.1	66.104	0.059	0.0892533	
	18.6	15.5	67.64	0.005	0.0073921	
	17.6	14.5	48.123	0.004	0.008312	
	19.6	16	76.311	0.009	0.0117938	
	18	14.8	66.493	0.005	0.0075196	
	18.9	15.7	75.594	0.009	0.0119057	
	19.2	16.2	82.801	0.01	0.0120771	
	19.5	16	72.857	0.014	0.0192157	
	19.6	16.2	80.271	0.013	0.0161951	
February	20.7	17	96.17	0.051	0.0530311	0.0459 (± 0.009)
	20	16.5	84.962	0.049	0.0576728	
	21.5	18.4	105.525	0.037	0.0350628	
	21.2	17.3	102.037	0.047	0.0460617	
	24	20.4	138.1	0.052	0.0376539	
	17.9	14.6	62.23	0.017	0.027318	
	20.2	16.5	84	0.018	0.0214286	

March	20.2	16.9	88.348	0.043	0.0486712	0.03829 (± 0.012)
	18.5	15.5	71.945	0.023	0.0319689	
	18	14.8	58.678	0.024	0.0409012	
	19	16.1	70.235	0.033	0.0469851	
	20	16.5	88.52	0.033	0.0372797	
	17.3	14.5	54.788	0.033	0.0602322	
	19.8	16.3	80.478	0.024	0.0298218	
April	15.9	13.3	47.396	0.009	0.0189889	0.02046 (± 0.014)
	19.1	15.9	67.211	0.007	0.010415	
	16.3	14.1	61.58	0.007	0.0113673	
	19.3	15.6	67.598	0.034	0.0502973	
	18.6	15.4	68.619	0.019	0.0276891	
	16.7	13.8	49.452	0.008	0.0161773	
	19	15.5	72.141	0.006	0.008317	

Table-04: GSI of female *Channa punctatus* in different months. Each value represents Mean ± SD.

Month	TL (cm)	SL (cm)	TBW (g)	GW (g)	GSI	Mean GSI
May	18	15	57.988	4.285	7.3895	7.8705 (± 1.034)
	19	13.7	41.941	3.8	9.0603	
	17.9	14.9	45.91	3.288	7.1618	
June	19.5	16	58	4.605	7.9397	6.5312 (± 1.284)
	18.9	15.6	53.714	3.346	6.2293	
	22.2	18	79.49	4.312	5.4246	
July	18	15.5	58.238	1.044	1.7926	2.5658 (± 1.006)
	16	13.8	43.097	1.596	3.7033	
	16	13.5	47.148	1.038	2.2016	
August	19.4	15.1	51.38	2.087	4.0619	5.0376 (± 2.173)
	18.1	14.8	44.776	1.446	3.2294	
	15.5	12.8	36.514	2.984	8.1722	
	15.9	13	42.715	2.002	4.6869	
September	16.4	14	42.636	0.055	0.1290	0.9201 (± 1.333)
	17.8	14.9	51.908	1.021	1.9669	
	16.2	13.2	41.261	0.021	0.0509	
	15.2	12.8	34.468	0.185	0.5367	
	14.3	11.6	26.723	0.012	0.0449	
	16.8	14.1	44.59	0.431	0.9666	
	12.8	10.6	19.492	0.029	0.1488	
	16.9	14.3	46.887	1.349	2.8771	
	16.5	13.9	44.916	0.034	0.0757	
	14.6	11.9	28.573	0.05	0.1750	
	19.1	15.6	30.062	1.209	4.0217	
	14.9	12.3	31.656	0.015	0.0474	
October	16.5	14.2	46.78	0.078	0.1667	0.5756 (± 1.163)
	15.3	12.8	37.215	0.047	0.1263	
	17.2	14.4	49.324	1.585	3.2134	
	22.5	19	124.61	0.111	0.0891	
	18	15	59.465	0.099	0.1665	
	18.8	15.7	67.671	0.08	0.1182	

	17	14.4	52.267	0.078	0.1492	
November	19.6	16.5	80.882	0.137	0.1694	0.2125 (± 0.081)
	16.2	13.4	39.067	0.118	0.3020	
	15.1	12.8	40.718	0.122	0.2996	
	16	13.2	43.588	0.058	0.1331	
	16.7	13.9	52.39	0.083	0.1584	
December	19.5	16	81.421	0.086	0.1056	0.2962 (± 0.438)
	17.6	14.1	57.532	0.043	0.0747	
	16.6	13.6	3.367	0.04	1.1880	
	18.7	15.8	70.018	0.092	0.1314	
	16.5	13.7	44.434	0.075	0.1688	
	15.8	13.1	39.541	0.043	0.1087	
January	17.2	14.4	58.126	0.165	0.2839	0.2580 (± 0.104)
	19.4	15.4	56.108	0.058	0.1034	
	17.5	14.6	66.124	0.216	0.3267	
	18.7	15.4	74.508	0.237	0.3181	
February	22.5	19.2	134.353	0.217	0.1615	0.2549 (± 0.106)
	23.1	19.1	139.221	0.23	0.1652	
	19.6	16.3	82.366	0.275	0.3339	
	21.3	18	110.9	0.398	0.3589	
March	17.4	14.7	65.506	0.549	0.8381	2.5942 (± 1.06)
	18	15.2	64.524	1.553	2.4069	
	17	14	55.745	1.669	2.9940	
	16.6	13.8	57.577	1.87	3.2478	
	18.6	15.8	78.722	2.743	3.4844	
April	17	14	52.544	1.021	1.9431	1.8527 (± 1.028)
	18.3	15.2	63.961	1.71	2.6735	
	16.8	14	49.976	0.251	0.5022	
	20.2	16.5	90.581	1.701	1.8779	
	16.9	13.9	56.264	1.697	3.0161	
	17.7	14.6	65.369	0.542	0.8291	
	19.2	16.1	80.91	2.507	3.0985	
	17.8	15	64.355	0.567	0.8811	

Chapter: Two

Biochemical comp0stion of *Channa punctatus* (Protein, Lipid, Ash & Moisture)

1.0 Introduction

Bangladesh is one of the populous country of the world. Here population is increasing day by day at an alarming rate. As the days proceed, the nutritious foods are decreasing with increasing of population. About 75% people of Bangladesh have been suffering from malnutrition. To over come this problem it is necessary to supply protein rich diet to the people and fish may be the important source of protein supply. Like other countries of the Third World, Bangladesh today is faced with the severe and widespread protein-calorie malnutrition problem resulting from low per capita consumption of protein on one hand and low intake of food (Chowdhury, 1981). At present, fish consumption per capita per year is only 12 kg, whereas the minimum requirement is about 18 kg for normal growth (DOF, 2002). The source of animal protein from land and water is decreasing day by day due to various natural and manmade factors.

Hossain *et al.* (1994) mentioned that among the fishing communities small fish occupy an important position as a popular food item. Small indigenous fishes have a high nutritional value in terms of both protein content and presence of micronutrients, vitamins, and minerals (Akhteruzzaman *et al.,* 1997). They contain large amount of calcium and most likely also iron and zinc (Tripathi, 1997). But over the last several decades the supply of fish had been declining and fish was becoming scarce day-by-day. As a result daily per capita consumption of fish has dropped.

Biochemical composition is the most commonly used system for evaluating the nutritional state of organisms or feeds. The composition of food gives its potential value. The normal composition of an animal is a useful base line for comparison in establishing the state of animal's health and nutrition (Hurwitz and Plavanik, 1986).

Fish is an excellent source of animal protein (Pearson & Muslemuddin, 1968). The main components of fish are protein, fat, ash, moisture and carbohydrate. These components play a vital role in evaluating the quality of the fish. An understanding of the nutritional aspects of fishes can lead to better utilization of their resources.

Chemical composition of fish is affected by numerous factors either of intrinsic in nature or environmental. Sexual stages of development influence the composition of fish (Legendre, 1938). The chemical composition of fish can be expected to vary largely from one species to another and within the same species from one individual to another (Jaquot, 1961). Variation in composition due to age difference of fish was also observed by Khuda *et al.* (1962). Many authors have recorded the seasonal effects of the chemical composition in fishes (Jaquot, 1961; Jafri, 1969).

Although a lot of works have been done on composition of different fishes but no report is available on the biochemical composition of *C punctatus* except that of Rahman *et al.* (2002). Their experiment showed the biochemical composition of *Channa punctatus* only in the body flesh rather than size, sex, parts of the body and annual variation. So, the present study was designed to assess the nutritive value in the body parts, size classes, various conditions (body flesh, sun drying, oven drying, boiled fish, fried fish, freezed fish and fish eggs) and the variation in nutrient content due to sexual stage differences and monthly variation of *C punctatus.* The present study was undertaken to obtain measurements of biochemical composition and seasonal changes in body composition of brood *C punctatus* and, in particular, to asses protein, lipid, ash and moisture.

Objectives

The present investigation was carried out to determine the biochemical composition of *C punctatus* with the following objectives:

I. To determine the proximate composition of different size class.

II. To estimate the proximate composition at different parts of the fish.

III. To assess the proximate composition of different conditions.

IV. To observe the seasonal variation of proximate composition.

2.0 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 little or no published information available on the different aspects of biology and biochemical composition of *Channa punctatus* (Bloch). The following information was reviewed in favor of the present study that was performed elsewhere in the world and relevant to the study.

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

Agarwal *et al.* (1972) studied the moisture content and gave an idea of self-stability of white pomfret, *Stromateus cinereus*. They recorded 72% of moisture in fresh fillets of white pomfret.

Stirling (1972) worked for determining of moisture, protein, ash and non-protein nitrogen in the white muscle, dark muscle, liver and gonads of European bass from the Bay of Naples. He stated that the white muscle has a food value, being rich in protein and low in lipid.

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

Dyer *et al.* (1961) studied on the proximate composition in various portions of the edible flesh of Atlantic halibut (*Hippoglossus hippoglossus*) and concluded that it varied greatly, especially in lipid content.

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) reported 5.30% to 9.50% moisture content in commercially important fresh water dry fish. Mannan (1977) found 49.5% moisture in *Hilsa ilisha.*

Khan *et al.* (1997) worked on quality aspects of some exportable dried fishery products of Bangladesh and reported that moisture, crude protein, lipid and ash content ranged from

16.20-23.51%, 58.37-82.86%, 0.17-14.44% and 0.58-9.23%, respectively.

Hossain *et al.* (1999) was analyzed the proximate composition of 23 small indigenous fish species and reported that the moisture content of different species ranged between 71 and 81.94%. The mucle 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.

Puwastien *et al.* (1999) were determined proximate composition and non-protein nitrogen of 8 fresh water and 8 marine fish species which are commonly consumed in Thailand. All fresh fish investigated were high in protein, 17-22 g/100g. Wide variation in protein content, 16-32 g/100g between species and methods of cooking was observed. Fat content varied between species; fresh water fish, 0.6-14 g/100g and marine fish, 0.5-9.2 g/100 g.

Shearer *et al.* (1994) reported that the parameters changed in relation to life cycle stage and fish size, and were influenced by whether the fish were in salt or fresh water.

Jonsson *et al.* (1998) reported that the lipid concentrations were highest in parr in the autumn. The relative water content decreased with age in parr in the autumn and increased with in smolts mean values being slightly higher in smolts (78%) than in parr (77%). Protein and carbohydrate concentrations did not vary with age in the immature 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.

3.0 Materials and Methods

For the present study, a total of 72 brood specimens of *Channa punctatus* were randomly collected during the month of May 2002 to April 2003 from the local market, Gallamary Bazar, Khulna. Among them 36 were male and 36 were female. The experiment was conducted in the Fish Nutrition Laboratory of Fisheries and Marine Resources Technology Discipline, Khulna University, Khulna.

Fishes were firstly cleaned by blotting paper and 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 electronic balance (B-303S). The length and weight was recorded in the nearest centimeter and gram respectively.

Biochemical composition

Biochemical composition of fish was determined in two ways i.e. wet weight basis and dry weight basis. In case of wet weight basis fish flesh was taken from three male species and three female species of *Channa punctatus* separately. The percentage of biochemical composition (protein, fat, ash and moisture) was determined for each species. Then the mean percent value of each biochemical component of male and female species was calculated separately.

In case of dry weight basis whole fish of male and female were taken and weighed. Then the fishes were placed in an oven (N4OC) at 105^0 C for 24 hrs. After drying they were weighed again and data were recorded. Fish were ground to powder the percentage of biochemical composition (protein, fat, ash and moisture) was determined for each species. Each experiment represents three replications.

Size classes: The fish were collected randomly from the local market and classified on their length basis. Three size classes were accounted viz. small size (7-9 cm), medium size (9-11 cm) and large size (≥11 cm) and the proximate composition was determined for each size class.

Body parts: The fish were collected from the local market in the month of May and carried to the Fish Nutrition Laboratory for dissection. Each fish was dissected into three parts viz. head, middle and tail. Each portion of fish species was analyzed to determine the proximate composition.

Different conditions: The fish conditions like fresh fish flesh, sun dried, oven dried, boiled fish, fried fish, frozen fish and fish egg were considered for proximate composition analysis. Experiment on each condition of the sample was completed in the month of October to determine their nutritional value.

Fish flesh: The collected fish was washed with clean water and scales, fins and internal organs were removed properly. The flesh of fish was cut from the body with the help of sharp sculpel scissors. Then these samples were prepared for analysis.

Sun dried fish: The collected fish was washed with clean water and weighed. Then this fish was placed in the open air for 3 days to dry properly. Before and at the end of sun drying their weight was recorded.

Oven dried fish: The collected fish was washed with clean water and weighed. Then this fish was placed in the oven at $105^{o}C$ for 24 hours. Their initial and final reading was recorded.

Boiled fish: The collected fish was cleaned properly. The scales, fins and internal organs were removed and boiled with fresh water. The weight of the fish was recorded before and after boiling.

Fried fish: The collected fish was cleaned properly and weighed. The scales, fins and internal organs were removed and fried with soybean oil.

Frozen fish: The collected fish was washed thoroughly clean water and excess water was removed by blotting paper and then placed it into a deep freeze that was kept at $-18^{o}C$ for 3 days.

Fish egg: The collected fish was dissected and eggs were removed with the help of a forceps.

In every case three replications were taken for accuracy. Standard deviation was calculated from three replications of each sample.

Analytical techniques:

Protein

Reagents and its preparation

1. Resolvent: Potassium sulphate and mercuric oxide at the ratio of 10 to 0.7 were mixed and preserved in colored bottle.
2. (98%) 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 or zinc powder.
6. 33-40% Caustic soda: 400 gm NaOH and 10 gm potassium sulfide was added with 1 liter distilled water.
7. 0.1 N HCl Acid: 8.48 ml HCl was added with 1 liter distilled water.

Digestion

0.2-0.5 g of sample was weighed and inserted into a Kjeldahl flask and 2 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 the 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. Then adding 70 ml of distilled water diluted the digested materials 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 sidewall. The flask was then 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.

Titration

The distilled solution was taken and titrated against 0.1 N HCl solution.

Calculation

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

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

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

Lipid

Fat was extracted with acetone by Soxhlet extraction and the extract thus obtained is weighed after careful recovery of the solvent.

Apparatus

- Soxhlet fat extractor (SF-6)
- Extracting thimble
- Hot plate (SH-1)
- Electronic balance (B303 S)

Chemical

- Acetone

Procedure

First of all weight of the empty thimble was taken. Then it was weighed with the sample. Difference between two weights gave the weight of the sample. Then the sample was placed in Soxhlet fat extractor apparatus. 70 ml of acetone was used and fat extracted with acetone. The apparatus was heated at 105^0 C for about 6 hours. After extraction of fat was placed on the hot plate (SH-1) until the acetone was completely evaporated.

Calculation

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

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

Moisture

Moisture was determined by complete drying of the sample at 105^{0} C. The loss in weight of the sample is the measurement of moisture content.

Apparatus

- Oven (N40C)
- Porcelain crucibles
- Electronic balance (B303 S)

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 the weights gave the weight of the sample. Now the crucible with sample was put in a controlled oven and was dried at $105\ ^{0}$C for 24 hours. 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).

$$\% \text{ Moisture} = \frac{\text{Weight of the sample (g) - weight of the dried sample (g)}}{\text{Weight of the sample (g)}} \text{X100.}$$

Ash

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.

Apparatus

- Muffle furnace (S-33 6 RB)
- Porcelain crucible and desiccators.

Procedure

Firstly clean porcelain crucibles were heated in a muffle furnace at 600 ^{0} C and the crucibles were cooled and weighed and heating, cooling and weighing are repeated until constant weights of the crucibles were obtained. The sample with the crucible was weighed and recorded. After that, each crucible with the sample was placed in the muffle furnace. The samples 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 (Maynard, 1970 and Pearson, 1976).

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

4.0 Results

The present study was undertaken with a view to study the biochemical composition (protein, lipid, ash and moisture) of *C punctatus.* The percentage of protein, lipid, ash and moisture were calculated from their respective size class, body parts, different conditions, body flesh of both sexes (wet weight basis) and whole fish of both sexes (dry weight basis).

4.1 Size class:

Three size classes of fish were taken for the present study i.e. small size (7 to 9 cm), medium size (9 to 11 cm) and large size ($\geq$ 11 cm). Knowledge of variation in biochemical composition in different stages of growth is essential as it enables exploitation of the fish when their nutritional value is higher. The results of biochemical composition of *C punctatus* have been presented in Table- 05.

The protein content was highest in the large size fish (19.37$\pm$ 0.21) and lowest in the small size fish (17.77 $\pm$ 0.14) (Fig-08). Lipid content was highest in the large size fish (1.10 $\pm$ 0.03) while lowest in the small size fish (0.07$\pm$ 0.14) (Fig-09). The mean value of ash content was highest in the medium size fish (2.45 $\pm$ 0.39) and lowest in the large size fish (1.75 $\pm$ 0.02) (Fig-10). The average moisture content was highest in the small size fish (82.73 $\pm$ 1.03) and lowest in the large size fish (79.64 $\pm$ 0.10) (Fig-11). The highest value of protein and lipid content was observed in large size fish and the lowest value of ash and moisture content was observed in large size fish.

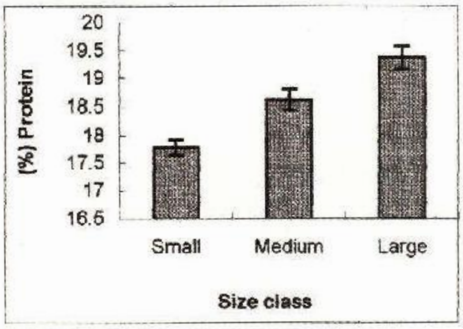


Fig-08: Protein content in various sizes of *C punctatus*. Error bar indicates standard deviation.

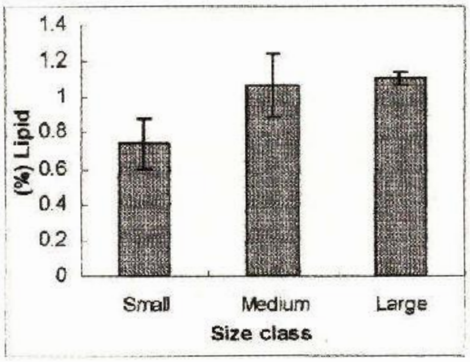


Fig-09: Lipid content in various sizes of *C punctatus*. Error bar indicates standard deviation.

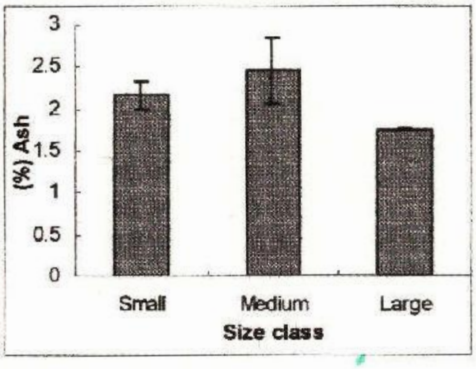


Fig-10: Ash content in various sizes of *C punctatus*. Error bar indicates standard deviation.

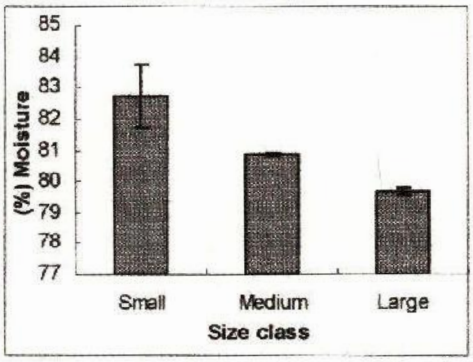


Fig-11: Moisture content in various sizes of *C punctatus*. Error bar indicates standard deviation.

4.2 Body parts:

For the present study, samples were taken from three parts of fish body (head, middle and tail) of both sexes. The results of proximate composition of *C punctatus* have been presented in Table- 06.

The protein content in the body parts varied from 17.35 ± 0.94% to 20.69 ± 0.89% and the highest value (20.69 ± 0.89%) observed in the middle portion of male and the lowest value (17.35± 0.94%) in the middle portion of female. In head portion, the protein content was higher in male than female. Incase of tail, the protein content was higher in female than male (Fig-12).

The lipid content in the body parts varied from 1.32 ± 0.02% to 2.34 ± 0.23% and the highest value (2.34 ± 0.23%) observed in the tail portion of male and the lowest value (1.32 ± 0.02%) in the head portion of female. It was found that lipid content was higher in different parts of the male than female (Fig-13).

The ash content in the body parts varied from 1.25 ± 0.007% to 3.54 ± 1.21% and the highest value (3.54 ± 1.21%) observed in the head portion of female and the lowest value (1.25 ± 0.007%) in the tail portion of male. The ash content was nearly similar in the middle portion (Fig-14).

The moisture content in the body parts varied from 76.31 ± 0.09% to 78.08 ± 0.19% and the highest value (78.08 ± 0.19%) was observed in the middle portion of female and the lowest value (76.31 ± 0.09%) in the head portion of male. Among the different portion of male fish, the moisture content was highest in tail and lowest in head and opposite result was observed for female (Fig-15).

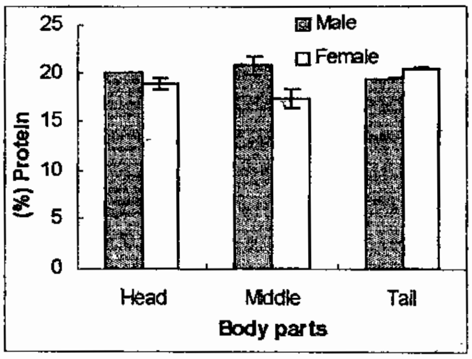


Fig-12: Protein content in various parts of *C punctatus*. Error bar indicates standard deviation.

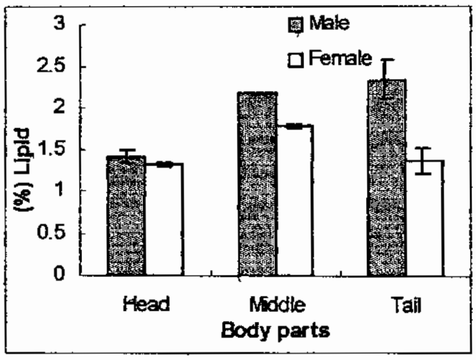


Fig-13: Lipid content in various parts of *C punctatus*. Error bar indicates standard deviation.

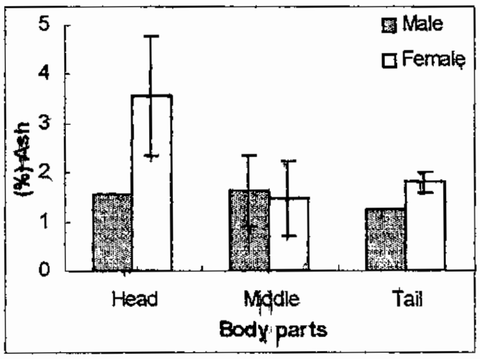


Fig-14: Ash content in various parts of *C punctatus*. Error bar indicates standard deviation.

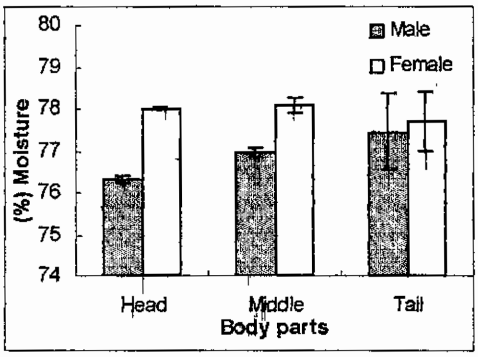


Fig-15: Moisture content in various parts of *C punctatus*. Error bar indicates standard deviation.

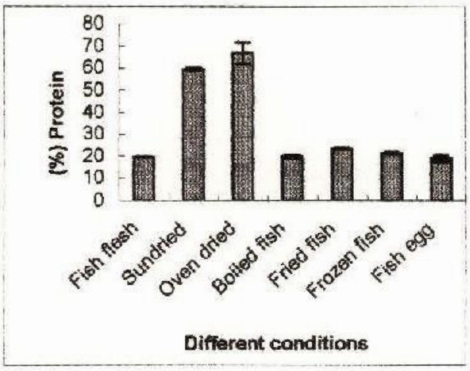
4.3 Different conditions:

Proximate composition of fish at different conditions i.e. fish flesh, sun dried fish (3 days), oven dried fish (24 hrs), boiled fish, fried fish (with oil), frozen fish (-18^0 C for 3 days) and fish eggs were taken for comparative study. The results of biochemical composition of *C punctatus* have been summarized in Table- 07.

In different conditions of fish, the protein content varied from 18.63 ± 1.29% to 66.51 ± 4.87%. The highest protein value was found in oven dried fish (66.51 ± 4.87%) and the lowest value in fish eggs (18.63 ± 1.29%). In general, dried fish contained more protein value (Fig-16).

In different conditions of fish, the lipid content varied from 0.65 ± 0.43% to 30.04 ± 1.40%. The highest lipid value was found in fish eggs (30.04 ± 1.40%) and the lowest value in boiled fish (0.65 ± 0.43%). In general, fish eggs contained more lipid value (Fig-17).

In different conditions of fish, the ash content varied from 1.20 ± 0.14% to 34.17 ± 1.29%. The highest ash value was found in oven dried fish (34.17 ± 1.29%) and the lowest value in boiled fish (1.20 ± 0.14%) (Fig-18).



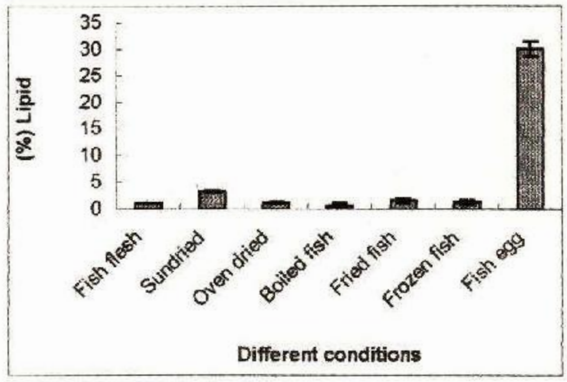


Fig-16: Protein content in various conditions of *C punctatus*. Error bar indicates standard deviation.

Fig-17: Lipid content in various conditions of *C punctatus*. Error bar indicates standard deviation.

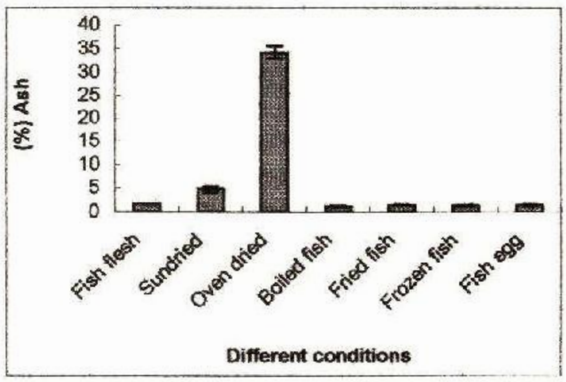


Fig-18: Ash content in various conditions of *C punctatus*. Error bar indicates standard deviation.

4.4 Seasonal variation in body flesh (on % wet- weight basis):

The body flesh of male and female *C punctatus* was taken to determine the month wise variation in proximate composition. Seasonal cycles of variation in the proximate constituents are generally attributed to the complex interaction between environmental parameters, food availability, growth and reproductive activity. The results of proximate composition of male and female *C punctatus* have been presented in Table-08.

Male

The protein content varied from 16.78 ± 1.26 to 22.34 ± 0.31% with maximum in November and minimum in July (Fig-19). Maximum lipid content observed in February (2.49 ± 0.17%) and minimum in July (0.76 ± 0.33) (Fig-20). The average value of ash content varied from 1.32 ± 0.02 to 2.12 ± 0.57% with maximum in September and minimum in August (Fig-21). The moisture content in the present study varied from 76.56 ± 0.18 to 81.36 ± 1.36% with maximum in August and minimum in November (Fig-22).

Female

The protein content varied from 18.00 ± 1.27 to 22.57 ± 2.02% with maximum in September and minimum in April (Fig-19). The maximum (2.26 ± 0.32%) and the minimum (0.91 ± 0.89%) lipid content were found in February and November, respectively (Fig-20). The average ash content varied from 1.16 ± 0.12 to 2.28 ± 1.04% with maximum in February and minimum in December (Fig-21). Moisture percentage was highest in the month of August (80.01 ± 0.14%) and lowest was in the month of February (77.03 ± 1.23%) (Fig-22).

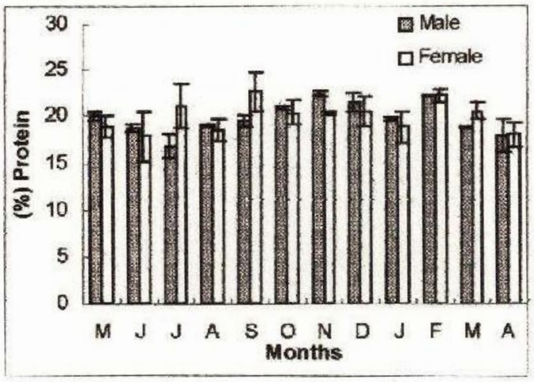


Fig-19: Monthly variation in protein content of male and female *C punctatus*. Error bar indicates standard deviation.

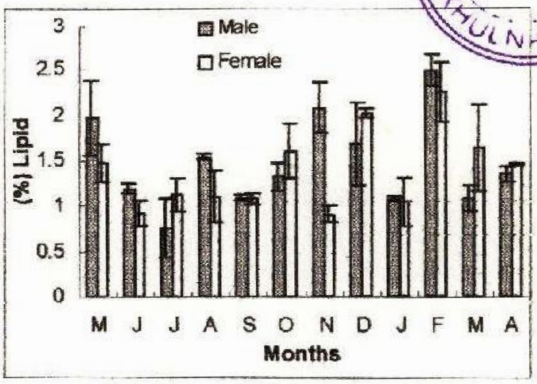
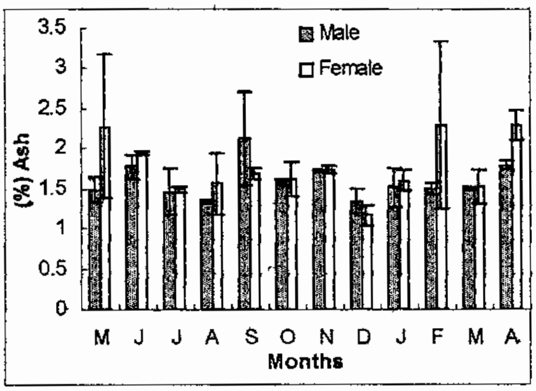


Fig-20: Monthly variation in lipid content of male and female *C punctatus*. Error bar indicates standard deviation.



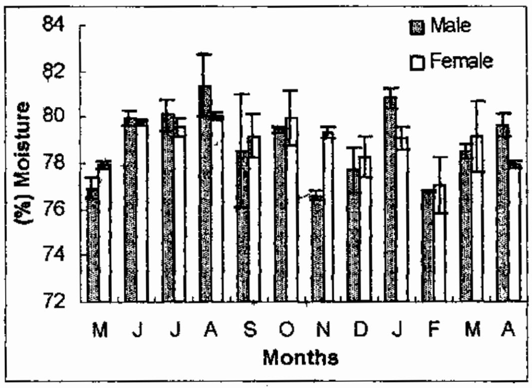


Fig-21: Monthly variation in ash content of male and female *C punctatus*. Error bar indicates standard deviation.

Fig-22: Monthly variation in moisture content of male and female *C punctatus*. Error bar indicates standard deviation.

4.5 Seasonal variation in whole body proximate composition (on % dry weight basis):

In dry conditions the whole body of male and female *C punctatus* was taken to determine month wise variation in biochemical composition. The results of biochemical composition of both sexes have been presented in Table-09.

Male

The average protein content varied from 51.08 ± 7.44 (April) to 78.67 ± 0.35% (January) (Fig-23). The lipid content varied from 1.23 ± 0.19 to 21.60 ± 0% with maximum in April and minimum in July, respectively (Fig-24). The mean ash was highest in July (34.17 ± 1.29%) and lowest in February (15.59 ± 0.73%) (Fig-25). Moisture content varied from 66.95 to 75.48% in month of February and November, respectively (Fig-26).

Female

The maximum protein content was found in September (76.13 ± 0.83%) and minimum in February (38.45 ± 5.8) (Fig-23). The lipid content varied from 2.13 ± 0.04 to 19.58 ± 0.12% with maximum in March and minimum in June (Fig-24). The mean value of ash content varied from 10.89 ± 2.1 to 28.59 ± 0.49% with maximum in June while minimum in February (Fig-25). The highest moisture content was found in October (76.53%) and lowest value in February (64.12%) (Fig-26).

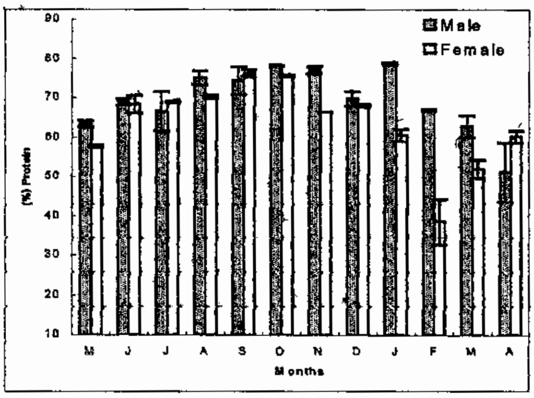


Fig-23: Variation in protein content of male and female whole fish (dry) *C punctatus*. Error bar indicates standard deviation.

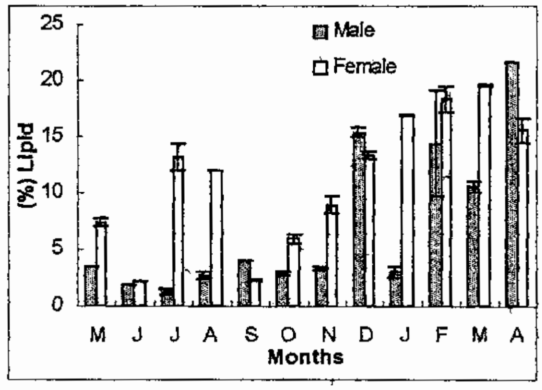


Fig-24: Variation in lipid content of male and female whole fish (dry) *C punctatus*. Error bar indicates standard deviation.

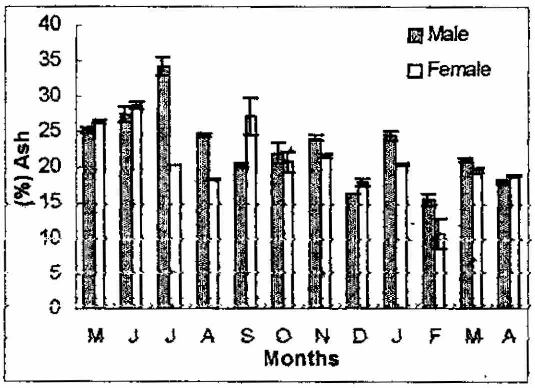


Fig-25: Variation in ash content of male and female whole fish (dry) *C punctatus*. Error bar indicates standard deviation.

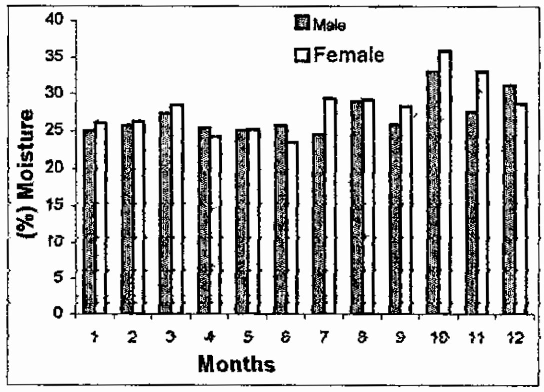


Fig26: Variation in moisture content of male and female whole fish (dry) *C punctatus*. Error bar indicates standard deviation.

5.0 Discussion

5.1 Size Class:

The protein content was highest in the large size fish (19.37 ± 0.21%) and lowest in small size fish (17.77 ± 0.14%). Shearer *et al.* (1994) found that protein concentration showed a gradual increase with growth, increasing from 12 to approximately 18% when the fish had reached 125g. Chowdhury (1981) found that large species exhibited the highest protein content (17.1%) while the lowest content (13.28%) was observed in small on. The protein content of gravid fish showed the highest value (19.37 ± 0.21%) and this highest value is expected, as the yolk is a reverse of lipo-protein to be utilized in the development of the embryo. This coincided with highest protein content found in ripe *Puntius filementosus* and *Gobioides rubicundus,* as reported by Vijaya Kumar (1987), Kader *et al.* (1984), respectively. Jansson and Jansson (1998) reported that young trout species use their surplus food consumption largely for protein production and little is stored reserve energy as their lipid contents are low. However, the lipid storage and energy concentration increase with the age of the brown trout in the autumn. This is consistent with the present investigation. By having a rapid protein growth, the young fish are gradually decreasing their vulnerability towards predations and increase their completion ability in situation of interference (Dill, 1983 and Magunam, 1988). Small fish use food for elevating growth. So, maximum protein in food is utilized for performance by younger species rather than mature or large species.

The lipid content was highest in the large size fish (1.10± 0.03%) and lowest in small size fish (0.73±0.14%). Shearer *et al.* (1994) found that lipid concentration increased in size up to 125g. He also observed that the level of lipid decreased during maturation. Levels of total fat within the samples were quite variable, as expected, since it is known that fat in fish varies by age, sex and reproductive status (Kinsella, 1988). Chowdhury (1981) found that large size of *C striatus* contained the highest value of fat (4.1%) while small size species contained the lowest value of fat (1.47%). The present investigation also favor the results obtained by Chowdhury (1981).

The lipid content in body parts varied from 1.32% to 2.34% and the highest value was observed in the tail portion of male and lowest value in the head portion of female. It was found that lipid content was higher in different parts of male than female that supports the result of Shearer *et al.* (1994) who explained that the level of lipid decreased during maturation. He also found that the parameters changed in relation to life cycle stage and fish size and were influenced by whether the fish were in salt or fresh water. Rahman *et al.* (1982) reported 1.02 ± 0.05% to 11.69 ± 0.26% crude fat in fresh water zeol fishes.

The ash content in body parts varied from 1.25 to 3.54% and the highest value was observed in the head portion of female and lowest value in the tail portion of male Shearer *et al.* (1994) reported that males contained higher concentrations of ash than females during maturation. The present study observed that head flesh contained highest level of ash than other parts of body due to a higher proportion of bones, gills and others. CSIR (1962) reported that the ash content of some selected fish species in India ranged between 1.53 and 2.60%. Rubi *et al.* (1987) found 0.83 to 1.76% ash in fresh muscle. Their findings were quite different from the present investigation, which could be due to sexual differences of species.

The highest value of moisture (78.08%) was observed in the middle portion of female and lowest value (76.32%) in the head portion of male. Belinsky *et al.* (1996) showed that moisture was quite consistent within fish sample part (i.e. flesh, liver and eggs). A moisture content of 77.24 to 82.12% was recorded by Gheyasuddin *et al.* (1980) in various fresh fishes. It may be due to inter specific species variation and sizes of fish.

5.3 Different Conditions:

The protein contents of fish flesh, sun dried fish, oven dried fish, boiled fish, fried fish, frozen fish and fish eggs were 19.37 ± 0.02, 59.34 ± 0.68, 66.51 ± 4.87, 19.59 ± 0.66, 22.96 ± 0.37, 21.11 ± 0.31 and 18.63 ± 1.29%, respectively. Among the different conditions of fish, the highest value of protein (66.51 ± 4.87%) was found in oven-dried fish and lowest value (18.63 ± 1.29%) in the fish eggs. Hossain *et al.* (1999) reported nearly similar protein content on the dry weight basis (69.81%) in *C punctatus*. Khan *et al.* (1997) reported 58.37 to 82.86% crude protein in some exportable dried fishery products. In contrast to the different samples, fish eggs had generally lower protein which was similar to the result of

Belinsky *et al.* (1996) on eggs of white fish (10.9/100g) and lake trout (19.6/100g). Puwastien *et al.* (1999) reported that boiled and steamed fish had a protein range similar to that of the fresh fish (16-24g/100g), which agrees with the present study. Tarr (1965) reported that some loss of organic nitrogenous constituents, largely sacro plasmic protein and inorganic salts with free drip are probable contributing factor of such loss of protein contents in chilled fish. Frying of fish that is more commonly practiced, resulted in amount of protein, ranging from 25.5-32g/100g (Puwastien *et al.*, 1999). The findings of Gall *et al.* (1983) where deep fried fish fillet had significantly higher protein than raw fillet.

The lipid content of fish flesh, sun dried fish, oven dried fish, boiled fish, fried fish, frozen fish and fish eggs was 1.10 ± 0.007, 3.22 ± 0.23, 1.23 ± 0.19, 0.65 ± 0.43, 1.55 ± 0.31, 1.26 ± 0.40 and 30.03 ± 1.40%, respectively. The highest lipid content was found in fish eggs (30.03 ± 1.40%) and the lowest value in boiled fish (0.65 ± 0.43%). Belinsky *et al.* (1996) reported that white fish egg conten 7-8/100g lipid. Puwastien *et al.* (1999) reported that various boiled and steamed fish contain the same range of fat as raw fish. He also reported that fried fish contained a higher level of fat compared to raw fish (8.5-22.7g verses 0.5-9.2g/100g) mainly due to absorption from cooking oil. Kamal *et al.* (2000) reported that at the end of 10 days of ice storage, lipid content of fresh water prawn slightly increased. But the lipid content in the present study was slightly increased which was frozen for three days. This difference may be due to duration of freezing time. The water content was gradually removed from the fish and concentration of other components viz. protein and lipid increased. Hossain *et al.* (1999) showed that the dried *C punctatus* contain 7.60 % lipid content, which do not support the result of the present study. Khan *et al.* (1997) reported that the lipid of some exportable dried fishery products ranged from 0.17 to 14.44% that agrees with the present findings.

The ash content of fish flesh, sun dried fish, oven dried fish, boiled fish, fried fish, frozen fish and fish egg was 1.75 ± 0.009, 4.74 ± 0.61, 34.17 ± 1.29, 1.20 ± 0.14, 1.52 ± 0.03, 1.46 ± 0.08 and 1.6 ± 0.08%, respectively. The highest ash content was found in oven dried fish (34.17 ± 1.29%) while the lowest value in boiled fish (1.20 ± 0.14%). Khan *et al.* (1997) reported that the ash content of some exportable dried fishery products of Bangladesh ranged from 0.58 to 9.23%. Hossain *et al.* (1999) found 22.34% ash content in the dried *C* *punctatus*. Puwstien *et al.* (1999) showed that sun dried and steamed short bodied mackerel resulted in a much higher ash content, 8.7 and 4.4 g/100g respectively. Belinsky *et al.* (1996) found that the ash content of flesh and eggs of white fish was 0.9±0.8 and 0.74g/100g, respectively. Kamal *et al.* (2000) reported that at the end of 10 days of ice storage, ash content of fresh water prawn slightly increased. There was a fluctuation in ash content with the present findings compared to others. It may be due to location, month of the year, size and sexes of fish.

5.4 Seasonal variation in body flesh (on% wet weight basis):

The protein content varied from 16.78 ± 1.26 to 22.34 ± 0.32% with maximum in November and minimum in July for male and for female, the protein content varied from 22.57 ± 2.02 to 18.00 ± 1.27% with maximum in September and minimum in April. The protein content of acetes shrimp varied between 14.72 ± 0.02 to 18.30 ± 0.03% with maximum in July and minimum in February (Ali, 2001). Banu *et al.* (1985) found 14.03 to 20.12% protein in various fishes and prawns. Mannan (1977) reported fish contain generally 18% protein. Hossain *et al.* (1999) reported that the muscle protein content among some small indigenous fish species of Bangladesh varied widely from 16.16-22.28% and *C punctatus* contained 17.15% protein. His reports corroborate with the present findings. Berg *et al.* (1998) reported that the protein content of young riverine Atlantic salmon and brown trout declined greatly in winter but were replenished rapidly in spring. Rates of decline were slower for the smallest fish, which also had the lowest specific content of protein while the differences in absolute amounts were greatest for the largest fish. Belinsky *et al.* (1996) reported that the protein content of female white fish, lake trout and brook trout was 15 ± 2.85, 15 ± 0.72 and 17.3g/100g, respectively. Shearer *et al.* (1994) revealed that the protein was observed to increase with fish size, but showed a slight decrease in early spring. This was attributed to reduce feeding during the winter and early spring. Jonsson *et al.* (1998) reported that protein concentration did not vary with age in the immature fish, mean protein concentrations being 18, 17.5 and 16.8% in parr in the autumn and spring and in smolts respectively. The variation of protein in the body flesh might be due to seasons, sex and size of fishes.

Maximum value of lipid content (2.49 ± 0.17%) found in February and minimum value (0.76 ± 0.33%) in July for male and maximum value (2.26 ± 0.32%) in February and minimum value (0.91± 0.08%) in November for female. Ali (2001) reported that the lipid content of acetes shrimp ranged from 1.47 ± 0.02 to 2.10 ± 0.01% with maximum in February and lowest in August. His result is nearly similar to the present investigation but the species was different and slight fluctuation occurred. The variation in fat content was influenced by the variation of species, diet, temperature, salinity selective mobilization and distribution (Lovern, 1950). Absar (2001) reported the highest value (2.32 ± 0.05%) of lipid in the edible portion of *Plotosus canius* occurred in February and lowest value (0.96 ± 0.04%) in September, which was nearly similar to the present study. Berg *et al* (1998) reported that the mean specific fat content was reduced by 45 to 70% during winter, relative to the pre winter period (September) and during spring the fat content was replenished rapidly, particularly in smallest salmon fry (a three fold increase from April to June). He also found the seasonal differences in specific energy largely reflected the seasonal fluctuations in fat content. The drop in lipid concentration appears to parallel an increased growth rate with a channeling of foods into protein (Hoar, 1976). Fat is an easily mobilized energy reserve that can be used for activity during spawning when the energy uptake is extremely low (Jonsson and Graven, 1985). Shearer *et al.* (1994) reported that percent lipid remained relatively constant during egg incubation but decreased between hatch and first feeding and the level of lipid decreased during maturation. Hossain *et al.* (1999) reported that the carcass lipid content varied between 1.87 and 9.55% and *C punctatus* contained 1.87% lipid. The present findings agree with the result of Hossain *et al.* (1999). Belinsky *et al.* (1996) reported that the lipid content of female white fish, lake trout and brook trout was 0.7 ± 0.39, 0.4 ± 0.17 and 0.92g/100g, respectively. Levels of total fat within the samples were quite variable, as expected, since it is known that fat in fish varies by age, sex and reproductive status (Kinsella, 1988). The present findings suggest that the variation of fat content of both sexes may be due to the same cases found by Kinsella, (1988).

Maximum value of ash content (2.12 ± 0.57%) found in September and minimum (1.32 ± 0.02%) in August for male and for female maximum value (2.28 ± 1.04%) in February and minimum value (1.16 ± 0.12%) in December. Ali (2001) reported that the ash content of acetes shrimp ranged from 1.60 ± 0.01 to 1.91 ± 0.08% with maximum in February and minimum in July. The ash values fluctuated very poorly except the month of August and December. During this time lower ash values were observed and it may be due to specimen's were young or spent. Kadar (1984) reported that ash content in *Gobioides rubicundus* was found 3.21 to 3.35%. Shearer *et al.* (1994) reported that the ash level decreased by about 50% by the time the fish reached 250g then increased prior to maturation. Males contained higher concentrations of ash than females during maturation. Hossain *et al.* (1999) reported that the ash content of *C punctatus* was 5.49, which did not support the present study. CSIR (1962) reported that the ash content of some selected fish species in India ranged between 1.53 and 2.60%, which agrees with the present study. Belinsky *et al.* (1996) reported the ash content of female white fish, lake trout and brook trout was 0.9 ± 0.18, 1.0 ± 0.07 and 1.2g/100g, respectively. Rubi *et al.* (1987) found 0.83 to 1.76% ash in fresh fish muscle.

Maximum value of moisture content (81.36 ± 1.36%) found in August and minimum value (76.56 ± 0.18%) in November for male while maximum value (80.01 ± 0.14%) in August and minimum value (77.03 ± 1.23%) in February for female. Ali (2001) reported that the water content varied from 75.52 ± 0.15 to 79.81 ± 0.04% with maximum in January and minimum in July. A moisture range of 77.24 to 82.12% was recorded by Gheyasuddin *et al.* (1980) in various fresh fishes. Fluctuation of moisture may be for the specimen's maturing, gravid and spent stage (Kadar, 1984) and also for the growth of the fish (Master and Magar, 1954; Senta *et al.*, 1944). Belinsky *et al.* (1996) reported the moisture content of female white fish, lake trout and brook trout was 79.0 ± 1.1, 80.0 ± 1.1 and 77.7g/100g, respectively. Hossain *et al.* (1999) found 75.43% moisture in the muscle of *C punctatus*. His result was within the range of the present results both for male and female species. Since the moisture has an inverse relation with size of the fish, the fishes in the present study being small showed comparatively higher moisture content than that obtained by Rahman *et al.* (1982) in small zeol fishes of Bangladesh (70.60-80.44%). Jonsson *et al.* (1998) found the relative water content decreased with age in parr in the autumn and increased with age in smolts, mean values being slightly higher smolts (78%) than in parr (77%). The variation in proximate composition could be occurred due to the influences of season as well as size or age, breeding and feeding behavior, hydrological parameters, diet etc.

5.5 Seasonal variation in whole body (on % dry weight basis):

Maximum value of protein content (78.67 ± 0.35%) found in January while minimum value (51.08 ± 7.44%) in April for male and for female maximum value (76.13 ± 0.83%) in September and minimum value (38.45 ± 5.85%) in February. Hossain *et al.* (1999) found 69.81, 63.60 and 71.91% protein in the dried fish of *C punctatus, Mystus vittatus* and *Ompok pabda,* respectively. Khan *et al.* (1997) stated that the crude protein range of some exportable dried fishery products from 58.37-82.86%. Chowdhury (1981) observed 63.2 ± 4.12 and 62.85 ± 4.59% crude protein in the dry condition of *C punctatus* and *C striatus,* respectively. The nearly same result was found in April for male but other results for female disagree with the present findings.

Maximum value of lipid content (21.60 ± 0.1%) found in April while minimum value (1.23 ± 0.19%) in July for male and for female maximum value (19.58 ± 0.12%) in March and minimum value (2.13 ± 0.04%) in June were noted. Hossain *et al.* (1999) found 7.6, 13.44 and 16.7% lipid in the dried fish of *C punctatus, Mystus vittatus* and *Ompok pabda,* respectively. Khan *et al.* (1997) stated that the lipid ranged of some exportable dried fishery products from 0.17-14.44%. Chowdhury (1981) observed 9.15 ± 0.76 and 9.76 ± 4.31% lipid in the dry condition of *C punctatus* and *C striatus,* respectively.

Maximum value of ash content (34.17 ± 1.29%) found in July while minimum value (15.59 ± 0.73%) in February for male and for female maximum value (28.59 ± 0.49%) in June and minimum value (10.89 ± 2.1%) in February were noted. Hossain *et al.* (1999) found 22.34, 20.92 and 11.02% ash in the dried fish of *C punctatus, Mystus vittatus* and *Ompok pabda* respectively. Khan *et al.* (1997) stated that the ash ranged of some exportable dried fishery products from 0.58-9.23%. Chowdhury (1981) observed 23.26 ± 9.30 and 21.53 ± 2.42% ash in the dry condition of *C punctatus* and *C striatus,* respectively. This variation could be due to size, unnecessary particles (fins, bones, gills etc) and maturation period of the species.

Maximum value of moisture content (75.48%) found in November while minimum value (66.95%) in February for male and for female maximum value (76.53%) in October and minimum value (64.12%) in February were noted. Hossain *et al.* (1999) found 75.43, 79.45 and 78.67% moisture in the dried fish of *C punctatus, Mystus vittatus* and *Ompok pabda,* respectively. Khan *et al.* (1997) stated that the moisture ranged of some exportable dried fishery products from 16.20-23.51%. Chowdhury (1981) observed 75.97 ± 4.28 and 76.59 ± 4.15% moisture in the dry condition of *C punctatus* and *C striatus,* respectively. It may be due the specimen's maturation, gravid and spent stage and also for the growth and size of the fish.

Table-05: Proximate composition in different size class of *C punctatus*. Each value represents Mean ± SD.

Size class	Size range (cm)	% Protein	% Lipid	% Ash	% Moisture
Small size	7-9	17.77 ± 0.14	0.73 ± 0.14	2.16 ± 0.17	82.73 ± 1.03
Medium size	9-11	18.64 ± 0.18	1.06 ± 0.18	2.45 ± 0.39	80.84 ± 0.04
Large size	≥11	19.37 ± 0.21	1.10 ± 0.03	1.75 ± 0.02	79.64 ± 0.10

Table-06: Proximate composition in different body parts of *C punctatus*. Each value represents Mean ± SD.

Sex	Parts	% Protein	% Lipid	% Ash	% Moisture
Male	Head	19.86 ± 0.02	1.41 ± 0.07	1.55 ± 0.01	76.31 ± 0.09
	Middle	20.69 ± 0.89	2.17 ± 0.005	1.61 ± 0.72	76.95 ± 0.12
	Tail	19.37 ± 0.07	2.30 ± 0.23	1.25 ± 0.007	77.43 ± 0.89
Female	Head	18.81 ± 0.56	1.32 ± 0.02	3.54 ± 1.21	78.02 ± 0.008
	Middle	17.35 ± 0.94	1.78 ± 0.19	1.47 ± 0.75	78.08 ± 0.19
	Tail	20.35 ± 0.04	1.34 ± 0.15	1.80 ± 0.19	77.67 ± 0.71

Table-07: Proximate composition in different conditions of *C punctatus*. Each value represents Mean ± SD.

Conditions	% Protein	% Lipid	% Ash
Fish flesh	19.37 ± 0.01	1.10 ± 0.007	1.75 ± 0.009
Sun dried (3 days)	59.34 ± 0.68	3.22 ± 0.23	4.74 ± 0.61
Oven dried (24 hrs.)	66.51 ± 4.87	1.23 ± 0.19	34.17 ± 1.29
Boiled fish	19.59 ± 0.66	0.65 ± 0.43	1.20 ± 0.14
Fried Fish	22.96 ± 0.37	1.55 ± 0.31	1.52 ± 0.02
Frozen fish (-18^{0}C)	21.11 ± 0.31	1.26 ± 0.40	1.46 ± 0.08
Fish egg	18.63 ± 1.29	30.03 ± 1.40	1.60 ± 0.08

Table-08: Proximate composition in body flesh of male and female *C punctatus.* (% Fresh matter basis) Each value represents Mean ± SD.

Months	% Protein		% Lipid		% Ash		% Moisture	
	Male	Female	Male	Female	Male	Female	Male	Female
May	19.97±0.54	18.84±1.22	1.97±0.40	1.48±0.21	1.47±0.15	2.27±0.90	76.90±0.45	77.92±0.18
June	18.61±0.41	17.85±2.69	1.19±0.05	0.92±0.14	1.76±0.16	1.93±0.01	79.94±0.32	79.78±0.12
July	16.78±1.26	21.01±2.38	0.76±0.33	1.13±0.18	1.45±0.29	1.48±0.03	80.08±0.68	79.51±0.40
August	19.02±0.06	18.45±1.24	1.55±0.03	1.10±0.28	1.32±0.02	1.55±0.39	81.36±1.36	80.01±0.14
September	19.47±0.61	22.57±2.02	1.09±0.02	1.08±0.05	2.12±0.57	1.67±0.07	78.52±2.50	79.17±0.92
October	20.84±0.20	20.32±1.33	1.33±0.15	1.61±0.29	1.57±0.03	1.61±0.20	79.43±0.10	79.96±1.22
November	22.34±0.31	20.36±0.19	2.08±0.28	0.91±0.08	1.72±0.01	1.73±0.04	76.56±0.18	79.31±0.25
December	21.46±0.94	20.48±1.55	1.67±0.45	2.01±0.05	1.33±0.15	1.16±0.12	77.67±0.94	78.25±0.91
January	19.69±0.20	18.78±1.69	1.07±0.03	1.05±0.26	1.51±0.24	1.59±0.13	80.80±0.47	79.03±0.47
February	22.18±0.15	22.21±0.67	2.49±0.17	2.26±0.32	1.49±0.07	2.28±1.04	76.71±0.03	77.03±1.23
March	18.79±0.02	20.55±0.81	1.08±0.14	1.65±0.47	1.49±0.02	1.51±0.20	78.47±0.29	79.13±1.50
April	17.89±1.73	18.008±1.27	1.35±0.08	1.47±0.01	1.78±0.05	2.29±0.18	79.60±0.51	77.92±0.15

Table-09: Proximate composition in whole body (dry) of male and female *C punctatus.* (% Dry matter basis) Each value represents Mean ± SD.

Months	% Protein		% Lipid		% Ash		% Dry matter basis	
	Male	Female	Male	Female	Male	Female	Male	Female
May	63.34±0.83	57.64±0.31	3.51±0.05	7.37±0.31	25.19±0.33	26.34±0.31	25.00	26.10
June	68.92±0.77	68.28±2.21	1.81±0.04	2.13±0.04	27.35±1.15	28.59±0.49	25.75	26.26
July	66.51±4.87	68.99±0.24	1.23±0.19	13.16±1.14	34.17±1.29	20.23±0.07	27.35	28.48
August	74.94±1.86	70.19±0.40	2.65±0.27	12.04±0.01	24.55±0.22	18.28±0.03	25.55	24.21
September	74.25±3.56	76.13±0.83	3.99±0.02	2.26±0.10	20.28±0.30	27.11±2.73	25.07	25.23
October	78.14±0.17	75.47±0.22	2.79±0.15	5.94±0.41	21.91±1.51	20.66±1.38	25.75	23.47
November	76.97±0.93	66.51±0.82	3.31±0.11	8.90±0.76	23.99±0.38	21.51±0.27	24.52	29.40
December	69.79±1.83	68.02±0.29	15.36±0.42	13.35±0.27	16.2±0.008	17.97±0.54	29.03	29.24
January	78.67±0.35	60.54±1.49	2.95±0.53	16.89±0.14	24.42±0.69	20.29±0.10	25.81	28.32
February	66.89±0.21	38.45±5.85	14.40±4.75	18.38±1.15	15.59±0.73	10.89±2.10	33.04	35.8 8
March	62.72±2.72	52±2.29	10.45±0.50	19.58±0.12	21.13±0.30	19.64±0.43	27.49	33.01
April	51.08±7.44	60.12±1.49	21.60±0	15.58±1.009	17.90±0.20	18.78±0.12	31.20	28.66

Chapter: Three

Conclusion, Reference & Appendix

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 snakehead fish,*C punctatus*. It is clear from the result of the present study and the discussion made so far that *C punctatus* 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, environmental condition. 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. Dried fish of *C punctatus* attained more protein content than wet fish. From proximate composition of *C punctatus,* 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 *C punctatus*. The data can also be used as a guideline for promoting fish consumption and further detailed studies.

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