IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Determination of Cholesterol, Lactic Acid, Pyruvic Acid, Ascorbic Acid, and Biochemical Parameters in Serum of the Clarias Batrachus Fish

Dr. Amit Kumar

University Department of Chemistry,
B. R. A. Bihar University, Muzaffarpur, Bihar, India.

Abstract: *For the development of aquaculture, a good knowledge of the mechanisms regulating the reproductive function in fish is required as such knowledge makes it possible to exercise varying degrees of control on the different stages of reproduction to adapt the techniques to fit fish culturist. Though, reproductive physiology has progressed considerably in recent years owing to the development of new & varied techniques (Billiard & Marcel, 1986; Sinha & Srivastava, 1991; Shankar Das and Kulkarni, 2007) but it needs more studies on the biochemical aspects especially the role of blood in fresh water fish. Thus, it appears that fluctuations in blood constituents especially the inorganic ions of the Indian fresh water fish with relation to their reproductive cycle have not been studied in detail, while among the physiological parameters; reproduction is one of the most important phenomena of an animal to continue its existence. It is also known that vitamins play some important role in the development of germ cells as both Vit 'B' & 'C' are concerned with maturation of oocytes. It has also been concluded that the yolk precursor (vitellogenin) are synthesized in liver & brought to the oocytes by blood (Bohemen et.al; 1981). As blood performs the vital function of nutrition & respiration to each and every tissue of the body, its (blood) constituents must take active role in such processes. Therefore, any variations in these constituents of blood even due to season would have some effect on the Gonadal cycle as well, but only a few work is available on some of these physiological parameters (Idler & Bitner 1958; Gill et. al., 1983; Singh, 1990, 1996; Panday et.al., 1993) and thus needs further studies. In this paper we determine Cholesterol, Lactic Acid, Pyruvic Acid, Ascorbic Acid, and Biochemical Parameters in Serum of the Clarias Batrachus Fish.*

Keywords: Protein, Glucose, Albumin, Globulin, Fish, Clarias Batrachus (Linn).

1. Introduction

The average day length and temperature were recorded after averaging the timing of Sun-rise and Sun-set of each day of the month and by averaging the temperature taken thrice on every third day of the month during the year 2014 respectively. The average rainfall data was obtained from the local Govt. Meteorological observations station at Pusa, Samastipur.

After 7 to 10 days of acclimation, the body weight and length of at least ten fish for each sex (male and female) were measured, followed by measuring the weight and length of testes & ovary of the fish. The average values thus obtained, were recorded for each month of the year. The Gonadal index (G.I,) and gonadosomatic index were calculated for all above mentioned fish individually using the following formulae and the average value was recorded for each month.

G.I. (gm/cm) = Weight of Gonad (gm)/Length of Gonad (cm)

$$\text{G.S.I.} = \frac{\text{Weight of Gonad (gm)}}{\text{Weight of Fish (gm)}} \times 100$$

The blood of male & female fish were pooled separately either by Caudal vessel or directly by heart puncture and serum were collected after proper clotting of blood & centrifuging the same. Accordingly, five sample of each sex were prepared for biochemical determination of various parameters.

To avoid diurnal variations in blood parameters, the serum was collected in the morning hours between 7.0 to 9.0 A.M. During blood collection, no anticoagulant or anesthesia was used, but haparinized capillary tubes were used for determining haematocrit value by micro-haematocrit centrifuge (REMI, INDIA) in which blood filled capillaries were centrifuged for 15 minutes at 8000 rpm & packed cell volume was noted.

Haemoglobin content was estimated by acid haematine method with the help of Haemometer (Germany), while total erythrocyte count was done with the help of Neubuar double Haemocytometer (Germany) using Hyme's solution. Mean corpuscular Hemoglobin (MCH) representing weight of hemoglobin contained in each RBC and Mean corpuscular Haemoglobin concentration (MCHC) representing the average concentration of haemoglobin in RBC, were calculated by using following formulae:

$$\text{MCH (pg)} = \frac{\text{Hb gm/litre of blood}}{\text{RBC No. x 106 cm}}$$

$$\text{MCHC (\%)} = \frac{\text{Hb gm/litre of blood}}{\text{Ht\%}} \text{ x 10}$$

For biochemical estimations, following methods for different parameters were followed as described in Natelson (1971), Oscer (1976), Verley et al. (1980) and also by using diagnostic kits available.

2. Expperimental

2.1 Determination of cholesterol in serum

Reagents used:

(i) Glacial acetic acid (A.R.)

(ii) Conc. H_2SO_4 (sp. Gr. 1.84) A.R.

(iii) **Stock kiliani's ragent:** 8 gm of ferric chloride ($FeCl_3.H_2O$) was dissolved in 100 ml of glacial acetic acid.

(iv) **Working kiliani's ragent:** It was prepared fresh on the day of expt, by diluting 0.5 ml of stock Kiliani's solution to 50 ml with glacial acetic acid.

(v) **Standard cholesterol sol. :** Standard curve was prepared by processing different known concentrations of standard cholesterol sol. as follows & O.D. thus obtained, were plotted against respective concentrations on a semi logarithmic graph paper.

Test tube	1	2	3	4	5
Std. cholesterol sol.	0	0.05	0.1	0.2	0.3 cc
Glacial acetic acid	0.4	0.35	0.3	0.2	0.1 cc
Working Kiliani's reagent	7.7	7.7	7.7	7.7	7.7 cc
Conc. of cholesterol	0	50	100	200	300 mg/dl.

Procedure:

0.05 ml of serum was added in 8.0 ml of dilute Kiliani's reagent in a centrifuge tube. Mixed & left for 10 minutes. Centrifuged for 10 minutes of 3000 rpm. Now, 3 ml of its clear supernatant was taken into a test tube as sample and 3 ml of glacial acetic acid was taken in another test tube as blank. To each tube, 2 ml of conc. H_2SO_4 was added slowly from the wall side of the tube. Mixed cautiously & left for 30 minutes. It was read at 530 filter in the colorimeter. The OD thus obtained, was read from the standard curve, the value was multiplied by 2 and recorded as mg cholesterol/dl of serum.

2.2 Determination of lactic acid in serum

Reagents used:

(i) Tungstic acid-Equal volume of 0.15N. H_2SO_4 (2.85 ml conc. H_2SO_4 diluted to 500 ml with distilled water) and 2.2% Aq. Sod. Tungstate solution was mixed on the day of expt.

(ii) 20% and 1% Aq. $CuSO_4$ sol.

(iii) Calcium hydroxide powder (AR): Several vials of 200 mg $Ca(OH)_2$ powder were prepared by weighing the powder and kept air tight for the expt.

(iv) Conc. H_2SO_4 (sp. Gr. 1.84) A.R.

(v) p-hydroxybiphenyl reagent-It was prepared by dissolving 240 mg p-hydroxybiphenyl in 1.5 ml of 5% Aq. NaOH sol. and diluted to 30 ml with distilled water. It was kept in a brown bottle in refrigerator. It is stable for several months.

(vi) Standard lactic acid (10 mg/dl). It was prepaed by dissolving 213 mg of anhydrous lithium lactate in 50 ml distilled water and after adding 0.5 ml of conc. H_2SO_4, the sol. was diluted to 1 liter with distilled water.

To obtain anhydrous lithium acetate, 5 ml of 85% lactic acid was mixed with 5 ml of distilled water, 2 drops of phenol red indicator was added followed by addition of 20% saturated sol. of lithium carbonate in slight excess, indicated by phenol red indicator. The sol. was then heated to boil, again some amount of saturated lithium carbonate sol. was added. Cooled and 20 ml of absolute alcohol was added slowly. After some time, crystals of lithium acetate formed, which was filtered, washed with Abs. Alcohol, dried at 110°C in a oven & kept in an air tight bottle in desicetor.

Procedure:

Two test tubes were taken. In first test tube (T), 0.05 ml of plasma was taken with 1 ml of distilled water. In second test tube (S), 0.05 ml of standard lactic acid sol. was taken with 1 ml of distilled water. To each tube, 1 ml of Tungstic acid was added, mixed & centrifuged. Now, 1.5 ml of aliquots were taken separately from each tube, 0.2 ml of 20% $CuSO_4$ sol. was added in each tube followed by mixing of 200 mg $Ca(OH)_2$ powder. Mixed vigorously for 3-4 minutes, allowed to stand for 6-8 minutes and then centrifuged at 3000 rpm for 10 minutes.

Again 0.5 ml of aliquot of each tube was taken separately and 1% $CuSO_4$ sol. was mixed, followed by adding 3 ml of

Conc. H_2SO_4. The solutions were mixed properly & heated at 100°C for 5 minutes. Cooled under tap water. Now 0.1 ml of p-hydroxy biphenyle reagent was added in each tube, mixed vigorously & heated at 100°C for 90 seconds. Cooled to room temperature and read at 530 filter using colorimeter against a blank consisting 1:1 dilution of tungstic acid and distilled water.

Calculation : Lactice acid (mg/dl) = $\frac{\text{O.D. of Test}}{\text{O.D. of Standard}} \times 10$

2.3 Determination of Pyruvic acid in Serum

Reagents used:

(i) 10% TCA

(ii) 100 mg 2, 4-Dinitrophenyle hydrazine dissolved in 100 ml of 2N HCl, filtered & stored in refrigerator.

(iii) 10% Aq. Sod. Carbonate.

(iv) 1.5N NaOH (6 gm NaOH + 100 ml Distt. Water)

(v) 2.5N NaOH (10 gm NaOH + 100 ml Distl. Water)

(vi) Standard Pyruvic acid sol. (107 mg of lithium pyruvate + 100 ml 0.1 NH_2SO_4).
1 ml standard was diluted to 100 ml with distilled water & stored in refrigerator.
This contains 0.01 mg Pyruvic acid/ml.

Procedure:

1 ml of serum was taken in a test tube containing 5 ml of 10% TCA. Mixed & transferred in a centrifuges tube (T). In another test tube (S), 1 ml of standard (diluted) pyretic acid was taken along with 5 ml of 10% TCA, mixed & transferred in another centrifuge tube. Both tubes were centrifuged. Now 3 ml of clear supernatant from each tube were taken separately in two test tubes and warmed to 25°C (a very slight warming). 1 ml of dinitropheny hydrazine sol. was added in each test tube and left for 5 minutes in room temperature to react. Now 3 ml of benzene was added in each tube and a steam of air was passed through the mixture for 2 minutes. After settling the mixture, lower layer was discarded with the help of a capillary tipped dropper. Thereafter, 6 ml of 10% Na_2CO_3 sol. was added in each tube and again air was passed for 2 minutes. After permitting the mixture to settle, 5 ml of aqueous lower layer was taken & kept in other test tubes. To this, 5 ml of 1.5 N NaOH sol. was added in each tube, mixed & left for 10 minutes. Both were read at 530 filters with the colorimeter using a mixture of 5 ml of 10% Na_2CO_3 & 5 ml of 1.5 N NaOH sol. as blank.

Calculation: $\frac{\text{O.D. of Test}}{\text{O.D. of Standard}}$ = mg pyruvic acid/dl of Serum

2.4 Determination of ascorbic acid in Serum

Reagents used:

(i) 5% T.C.A. (Ice cold).

(ii) 65% H_2SO_4 sol. (Ice cold) : 70 ml conc. H_2SO_4 + 30 ml distilled water.

(iii) Dinitrophenylehydrazine reagent: 200 mg 2-4 dinitrophenyl hydrazine was mixed with 10 ml of 9N H_2SO_4 (5 ml conc. H_2SO_4 + 15 ml of distilled water). To this, 25 mg of thiourea was mixed, To this, 3 mg of $CuSO_4$ was mixed & filtered through glass wool before use. This sol. is stable for only one week.

(iv) Standard ascorbic acid sol. (1 mg/100ml) 1 mg ascorbic acid (pure) was dissolved in 100 ml of distilled water.

Procedure:

0.2 ml of serum was mixed with 1 ml of 5% cold TCA followed by mixing of 1 ml distilled water & left for 15 minutes. To a second test tube (S) 0.2 ml of standard ascorbic acid sol. was taken and 0.8 ml of 5% T.C.A. was mixed. In a third test tube (Blank), 0.5 ml of 5% TCA was taken. Ist (T) & 2nd (S) test tubes were centrifuged and filtrates were taken separately for further analysis.

1.5 ml of the filtrate of 1st test tube (T) was taken and to this 0.6 ml of dinitrophenyle hydrazine reagent was added, corked & incubated for 3 hours at 37°C along with the remaining two test tubes in which the 2nd tube (S) contained a mixture of 0.5 ml of filtrate and 0.2 ml of dinitrophenyle hydrazine & the third tube blank contained 0.5 ml TCA + 0.2 ml dinitrophenyle hydrazine reagent.

After incubation test tubes were chilled in ice bat. Now 2.4 ml of 65% H_2SO_4 was added separately in first (T) & second (S) test tube while 0.8 ml 65% H_2SO_4 was added in third (blank) test tube. Mixed with and left for 30 minutes. They were read at 530 filters using the colorimeter.

Calculation: $\frac{\text{O.D. of Test}}{\text{O.D. of Standard}} \times 1$ = mg ascorbic acid/dl of Serum

The fish under study, *Clarias batrachus* (Linn.), commonly known as "MAGUR" does not show any difference in their colour pattern between male & female. However, during June to August the belly (abdomen) of female became large rounded due to mature ovary, whereas, the males remain sleek and slender. If pressed laterally, they (female) showed protruded vent and eggs came out. In females the urinogenital papilla is short with large broad base, whereas in males the papilla is prominently large and elongated in sexually mature condition. Colour is uniform brown or grayish black. Males are smaller than females and number of males is less than females.

The average water temperature, day length and rainfall during different months of the year, 2014 and during different phases of the gonadal cycle of the fish were recorded and are summarized in Table 1 A & B whereas average body length and weight, of the fish, gonads (Testis & Ovary) length & weight, average gonadal index & gonadosomatic index are

presented in Table 2A & 2B .

The average day-length indicates that it is more than thirteen hours during May to July followed by 12.59 hr. in April & 12.56 hr. in August which coincides with the high water temperature (29.75° to 31.05° ±4.96°C) between May to July, followed by 28.4±8.97°C & 29.07±2.61°C in April & August respectively. The lowest water temperature & day length were recorded in December to February i.e. 14.48 to 16.66±5.46°C & 10.36 to 11.20 hours respectively. The average rainfall has been recorded highest in August (26.46±9.94 mm) followed by June (20.57±9.63 mm) and July (6.72±5.08 mm) respectively, where as in September & October it was recorded to 4.04±2.73 and 4.36±1.09 mm respectively. January, March, April, November and December months witnessed no rain at all and below 4.00 mm in rest of the months. Thus, the results indicate that the water temperature decreases with the onset of monsoon.

The average weight of the testes was found lowest in the months of November to January as it varied between 1.8±.02 to 2.4±0.04 gm. It gradually increases from February (2.9±0.06 gm) to May (3.5±0.05 gm) followed by a sharp increase in June (4.8±0.07 gm) and August (3.9±0.04). Thereafter abruptly declined to 2.6±0.02 gm in September & October (2.5±0.03 gm).

The average weight of ovary also showed almost similar trend as found in the testes. The lowest weight of the ovary was recorded in January (3.2±0.12 gm). It gradually increases up to 5.8±0.014 gm in March, followed by an abrupt increase in weight from April (6.7±0.10 gm) to June (12.85±0.20 gm). Thereafter it declined to 6.8±0.09 gm in July & continued upto December (3.6±0.09 gm).

Accordingly, the length of the gonads (both testes & ovary) also changed as it was recorded lowest in January (5.57 ±0.02 cm for testes & 5.94±0.03 cm ovary) and highest in December (12.10±0.05 cm) for testes & June (9.51±0.06 cm) for ovary.

The gonadal index was calculated to be lowest in November to January i.e. 0.15±0.003 gm/cm for male in December while it was 0.54±0.003 gm/cm in January for female fish, whereas maximum value was obtained in August 0.75±0.003 gm/cm for male & 0.89±0.004 gm/cm for female in July. Similarly, the G.S.I. for male was recorded lowest in November/December (1.8 to 2.08%) and in January 2.34% for female.

Thus, the weight of the testes & ovary, length of the gonads (both testes & ovary), ovarian-index and G.S.I. show parallelism in their activities as with the increase of gonad weight, all these parameters also increases.

Total erythrocyte content (TEC): The total RBC count in males was found always higher than the females, but in both sexes, it was recorded lowest in the month of December (3.40±0.05 and 2.89±0.06X10^6 m/cm in male & female respectively). It gradually increased and became maximum (i.e. 3.90±0.07x10^6 m/cm) in July and (3.70±0.06x10^6 m/cm) in August month in male & female respectively.

The females have significantly low RBC number ($P<0.05$) during resting, preparatory & prespawning phases when compared with the value obtained for their males counter parts. However, no significant difference was observed in total RBC counts between males & females during spawning & post-spawning phases though the female have comparatively lower value then the males as represented.

Among the males no significant increase was observed during preparatory and post spawning phases, but the increase was found satistically significant at 5% level ($P<0.05$) during pre-spawning & 1% level ($p<0.01$) during spawning phases when compared with the value of resting phase. Similarly, in females, the TEC gradually increased, which became significant ($P<0.01$) during pre-spawning & spawning phases while 5% ($P<0.05$) level during post spawining phase when compared with the value of resting phase.

MCH & MCHC : Both MCH & MCHC value in male & female fish showed a mixed trend as MCH was found higher in females than the males from November to May while it was higher in males from June to October. In male, the MCH value varied between 49.82 pg (in March) to 54.60 pg (in October) where as in female it varied between 49.58 g (in September) to 54.09 pg (in February).

Table 1: Seasonal variations in glucose, lactic acid, cholesterol & pyruvic acid in the serum (blood) of C. batrachus during the year 2014.

Month	Serum glucose (mg/dl)		Serum lactic acid(mg/dl)		Serum cholesterol(mg/dl)		Serum pyruvic acid(mg/dl)	
	Male	Female	Male	Female	Male	Female	Male	Female
1	2	3	4	5	6	7	8	9
Jan.	50.160 ± 2.040	53.720 ± 3.100	7.540±0.320	8.280 ± 0.240	310.700 ± 6.760	284.250 ± 7.100	0.175 ± 0.010	0.162 ± 0.006
Feb.	58.480 ± 2.120	64.540 ± 2.650	8.180±0.190	8.160 ± 0.170	326.450 ± 8.100	296.100 ± 6.320	0.183 ± 0.008	0.175 ± 0.009
Mar.	64.370 ± 3.100	71.230 ± 2.960	8.560±0.230	8.780 ± 0.150	357.800 ± 7.880	325.370 ± 8.400	0.194 ± 0.012	0.182 ± 0.010
Apr.	71.220 ± 2.680	76.650 ± 3.020	9.720±0.280	9.550 ± 0.220	365.160 ± 8.150	334.050 ± 7.260	0.212 ± 0.011	0.198 ± 0.012
May	82.750 ± 2.170	79.400 ± 2.680	11.400±0.250	10.230 ± 0.200	290.320 ± 6.670	280.800 ± 5.700	0.220 ± 0.013	0.204 ± 0.011
June	90.620 ± 2.720	85.910 ± 3.100	12.250±0.200	10.980 ± 0.230	276.740 ± 7.060	265.450 ± 6.650	0.226 ± 0.015	0.212 ± 0.010
July	102.790 ± 3.000	91.450 ± 2.260	13.020±0.290	13.760 ± 0.180	305.000 ± 8.520	268.920 ± 7.080	0.235 ± 0.012	0.220 ± 0.013
Aug.	112.940 ± 2.370	99.750 ± 2.700	10.860±0.240	13.180 ± 0.270	339.320 ± 6.250	286.750 ± 7.630	0.240 ± 0.014	0.216 ± 0.011
Sep.	87.380 ± 2.240	106.530 ± 3.050	9.350±0.150	11.000 ± 0.200	368.560 ± 8.960	338.140 ± 8.060	0.192 ± 0.010	0.234 ± 0.012
Oct.	74.420 ± 2.790	90.200 ± 2.580	9.700±0.220	10.230 ± 0.014	354.380 ± 9.320	343.550 ± 7.200	0.181 ± 0.009	0.220 ± 0.007

Nov.	55.250 ± 1.100	77.570 ± 1.960	9.160±0.190	9.460 ± 0.220	340.760 ± 7.850	318.900 ± 6.670	0.176 ± 0.010	0.179 ± 0.008
Dec.	48.700 ± 2.870	64.300 ± 2.020	8.280±0.270	8.150 ± 0.190	350.100 ± 5.200	300.540 ± 6.250	0.171 ± 0.012	0.168 ± 0.007

The MCHC% in males was recorded comparatively higher from April to November and varied between 34.33% (in June) to 35.81% (in October) and in females 32.70% (in June) to 34.44% (in August) However MCHC values were always found lower than the MCH in both sexes during the year of investigation.

Serum glucose: The serum glucose level in the male & female C. batrachus varied between 48.70±2.87 to 112.94±2.37 mg/dl and 53.72±3.10 to 106.53±3.05 mg/dl respectively between January to December months. In male lowest serum glucose level was recorded in the month of December. Thereafter it gradually increased & became maximum in August followed by a sharp decrease in September. In the female minimum value was recorded in January. It gradually increased to maximum in September followed by a sharp decline in November. However, the female have comparatively higher blood glucose level, than male from September to April (Table-1).

Accordingly, during present study, the spawning period of C.batrachus (both male & female) has been recorded during July & August (or late June to early August) Nayyar & Sundararaj (1970) have reported spermiogenesis in *H. fossilis* occurs during May-June & possibly cued by increasing temperature & photoperiod and spawning takes place during late June to August in Delhi, while Ghosh & Kar (1952) reported all through the year at Calcutta. The spawning period in female *H.fossilis* has been reported between July to August at Ripura (Nair, 1963), between July to September at Delhi (Viswanathan & Sundar raj, 1974) and between late July to September at Calcutta (Ghosh & Kar 1952). Thus, the difference in spawning periods may be due to some climatic factors such as geographical distribution (latitudinal or altitudinal) and/or commencement of monsoon/rainfall which could possibly highlight the individual environmental factors responsible for gonadal maturation & spawning. However, no serious attempt has so far been made to study the impact of geographical distribution of a species on their reproductive variations & hence needs further study on this aspect.

3. Conclusions

The fish *Clarias batrachus* (Linn) commonly known as “Magur” does not show any marked difference in their colour pattern between male & female but during breeding period *i.e.* from late June/July to August, the abdomen of female became bulged & pot-like in appearance anteriorly & if pressed laterally, eggs came out through the protruded vent, whereas, male had slender trunk region & possessed urinogenital papillae in sexually matured condition.

In the present study, lactic acid level in the blood of both male & female *C.batrachus* were recorded lowest during resting phase without any significant difference between the sexes but gradually increased significantly during pre-spawning with the maximum during spawning phase. Though, the lactate level in females was found significantly ($P<0.05$) lower than the value to male during pre-spawning phase, but a reverse was noted during spawning & post spawning phases. Thus, the increase decrease might be correlated with the activities of the fish during these phases of the gonadal cycle.

Robertson et. al., (1961) have reported that a correlation exists between blood cholesterol and one or more of the factors like age, sex, activity, diet, sexual maturity & spawning migration (especially in marine fish). Mc Cartney (1967) observed decreased serum cholesterol level at the time of maximum sexual activity in *Salmo tructta* and Katz & Eckstein (1974) correlated this situation with enhanced steroid genesis accompanying sexual maturation. According to Irvin et. al., (1957), the temperature tolerance of *Carassius auratus* can be extended by addition of cholesterol or phospholipids to the diet, but their effectiveness varies with the season, however, the mechanism is not known, Siddique & Naseem (1971) in *H. fossilis* and Naseem & Siddique (1971) in *C. mrigala* & *L. rohita* observed an increase in the plasma cholesterol level during the spawning & a decrease during the post-spawning periods. Tandan & Chandra (1976) in *Clarias batrachus*, reported an upward trend in blood cholesterol during pre & post spawning periods and hypercholesterolemia during spawning period.

Joshi et. al., (1979) have reported that cholesterol level generally depleted during winter, but in *C. batrachus Liver* & muscle showed a slightly elevated level. Bano & Hameed (1979) in *C. batrachus* and Singh (1990) in *C. fasciatus* observed almost an inverse relation between ovary & blood with liver & muscle and suggested the diversion of muscles cholesterol to the development of gonads in the form of sex hormones. Sex related cyclic changes in hepatic & serum cholesterol in fish have also been reported by Jaiswal et. al., (1978); Singh & Singh (1979); Joshi (1980) and Gill et. al., (1983). These authors reported that serum cholesterol declined during the spawning period, but increased during the post spawning period. According to Dietschy & Wilson (1970), the synthesis of cholesterol as well as its mobilization & utilization depends mainly on two physiological factors i.e., dietary intake & hormonal changes, and as the seasonal changes in the hormones are known to occur in migratory or seasonally breeding fish, it is not surprising that fluctuations in cholesterol level were due to changes in the dictary intake or in the circulating levels of hormones.

The serum cholesterol level was found maximum during post-spawning phase and lowest during spawning phase. Further, the females showed lower value of cholesterol content than the males. Thus, the hypercholesterolemia observed during pre spawning and spawning phases may be correlated with enhanced steroidgenesis accompanying sexual maturation and/or may be used as energy sources during spawning.

The pyruvic acid level in the blood of both male & female fish was recorded lowest during resting phase. It gradually increased & became statistically significant during spawning phase. However, maximum increase in male & female was observed during spawning & post spawning periods respectively. Further, the males have always higher values than the females except post spawning periods. Thus, the increase in blood pyruvic acid level may be due to sexual activities of the fish as it is normally produced upon the oxidation of lactic acid and is a normal intermediate in the metabolism of carbohydrate or fatty acids to carbon dioxide.

References:

1. *Agarwal, A. 1982: Studies on the karyotype & histological changes in the gonads of some fishes of Jammu & Kashmir, Ph.D. Thesis Univ. of Jammu.*
2. *Banerjee, S. and Banerjee, V. 1987 b: Erythrocytes and related parameters in Heteropneustes fossilis (Bloch) with special reference to body length, sex & season J. Adv. Zool., 8(2): 67-72.*
3. *Bentley, P.J. and Follett, B.K. 1965: Fat and carbohydrate reserves in the river lamprey during spawning migration. Life. Sci. 4 : 2003-2007.*
4. *Billard, R. and Marcel, J. 1986: Aquaculture of cyprinids. I.N.R.A., Paris.*
5. *Bohemen, C.G.; Lambert, J.G.D. and peute, J. 1981: Annual changes in plasma & liver in relation to vitellogenesis in the female rainbow trout, Salmo gairdneri. Gen. Comp. Endocrinol., 44: 94-107.*
6. *Chatterjee, P.K., Kumar, A. & Sinha, N.D.P. 1991 : Gonadal cycle & ovarian electrolyte behavior in air breathing teleost Channa punctatus (Bloch) and Heteropneustes fossilis (Bloch). J.Freshwater Biol. 3(a) : 37-44.*
7. *Dietschy, J.M. and Wilson, J.D. 1970 : Regulation of cholesterol metabolism. New. Eng. J. Med. 282 : 1128-1249.*
8. *Emmersen, B.K. & Patersen, I.M. 1976: Natural occurrence and experimental induction by estradiol 17-B of a lipopho-phoprotein (Vitillogenin) in flounder, Platychtys flessus L. Comp. Biochem. Physiol. 54(B): 443-446.*
9. *Gupta, S.K. 1996: Some histochemical & biochemical studies on the development of female reproductive organ of an air-breathing fish. Clarias batrachus (Linn.) and its relation with Thyroid follicles. Ph.D. Thesis, B.R.A.B.U., Muza., India.*
10. *Guraya, S.S.; Kaur, R. & Saxena, P.K. 1975 : Morphology of ovarian changes during the reproductive cycle of fish, Mystus tengara (Ham). Acta. Anat., 91 : 222-260.*
11. *Jaiswal, D.M.; Belsare, S.D. and Belsare, D.K. 1978: Correlative changes in hepatic and serum cholesterol during annual ovarian cycle in the Catfish, Heteropneustes fossilis (Bloch.). Bioresearch, 2(1) : 37-41.*
12. *Jonas, R.E.E. and MacLeod, R.A. 1960 : Biochemical studies on sockeye salmon during spawning migration X. Glucose, total protein, non-protein nitrogen and amino acid nitrogen in plasma. J. Fish. Res. Bd. Can. 17 : 125-126.*
13. *Joshi, B.D. 1980: Sex-related cyclic variations in blood glucose and cholesterol contents of a cat fish. Comp. Physiol. Ecol., 5(a): 13-16.*
14. *Joshi, B.D. 1982: Circannual fluctuation in some blood components of the fish, Rita rita in relation to certain ecophysiological conditions. Uttar Pradesh. J. Zool. 2 : 62-66.*
15. *Joshi, B.D. and Tandan, R.S. 1977: Seasonal variations in some haematological values of fresh water fishes I : H. fossilis and M. vittatus. Comp. Physiol. Ecol. 2 : 2-26.*
16. Irvin, D.G.; Newman, K. and Hoar, W.S. 1957: Effects of dietary phospholipid and cholesterol on the temperature resistance of gold fish. Can. J. Zool., 35: 691-709.
17. Jaiswal, D.M.; Belsare, S.D. and Belsare, D.K. 1978: Correlative changes in hepatic and serum cholesterol during annual ovarian cycle in the Catfish, *Heteropneustes fossilis* (Bloch.). Bioresearch, 2(1) : 37-41.
18. Katz, Y. & Eckstein, B. 1974: Changes in steroid concentration in blood of female *Tilapia aurea* during initiation of spawning. Endocrinology, 95% 963-967.
19. Kha, S.H. 1977: Studies on haematology of fresh water cat fish, *Clarias batrachus* (L.) I : Seasonal varations in erythrocytes and leucocytes counts. Comp. Physiol. Ecol. 2 : 88-92.
20. Khanna, S.S. and Singh, T. 1971: Studies on the blood glucose level in *Channa punctatus* (Bloch.). Acta. Zool. (Stockh.). 52: 57-101.
21. Loh, H.S. & Wilson, C.W.M. 1971: Relationship of human ascorbic acid metabolism to ovulation. Lancet, 1 : 110-112.
22. Love, R.M. 1980: The chemical biology of fishes. Academic Press. London
23. Malhotra, Y.R.; Jyoti, M.K. & Gupta, K. 1989: Reproductive cycles of fresh water fishes. In, “Reproductive cycles of Indian vertebrates (Ed. Saidapur, S.K.) pp. 58-105, Allied Publ. Ltd. New Delhi.
24. Malhotra, Y.R.; and Sharma, K.K. (1988): Importance of Calcium at breading sites of fishes, Current Sci. 57: 959-960.