haematological changes in two reproductive phases (pre-spawning phase and spawning phase) has been studied in the Indian fresh water fish, *N. notopterus*.

Materials and Methods

Fish samples

The live specimens of Indian fresh water fish, *Notopterus notopterus* were obtained from aquatic bodies situated in a local area in Gulbarga, Karnataka State, India during April month (pre-spawning phase) and August month (spawning phase). The fish were transported to laboratory in aerated container; they were given a minimum period of one week to acclimatize under laboratory conditions during which time they were fed with earthworms and boiled eggs.

Blood analysis

The haemoglobin content of blood sample was analyzed using cynamethemoglobin method described by (Baker and Silverton, 1982). Total serum proteins were measured by using modified Biuret method end point assay as described by (Lawrence , 1986).

Glucose was determined by GOD-POD method using commercial kit, urea & urea nitrogen was determined by using Berthelot method.

The erythrocyte count (EC) was determined in the Neubauer's haemocytometer following the method of (Wharton and McCarthy, 1972; Blaxhall and Daisley, 1973). The determination of haematocrit was carried out by Wintrobe method.

The haematological Indices: mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were calculated using the formula of (Baker and Silverton, 1982) given below:

Mean Corpuscular Volume (MCV fl)

$$= \frac{\text{Haematocrit (\%)} \times 10}{\text{Erythrocyte count (mm}^3\text{)}}$$

Mean Corpuscular Haemoglobin (MCH pg)

$$= \frac{\text{Haematocrit (g\%)} \times 10}{\text{Erythrocyte count (mm}^3\text{)}}$$

Mean Corpuscular Haemoglobin Concentration (MCHC) %

$$= \frac{\text{Haemoglobin (g\%)} \times 10}{\text{Haematocrit (\%)}}$$

Statistical Analysis

The statistical analysis of the data of intraspecies correlation 'r' of haematological and biochemical relationship were carried out by using statistical software SPSS 7.5.

Results

The haematological studies of fish *Notopterus notopterus* carried out under normal conditions are: erythrocyte count, haematocrit, haemoglobin contents, MCV, MCH, MCHC, protein, glucose, urea, urea nitrogen, creatinine, and cholesterol are presented in the table (1&2)

The reproductive cycle of the fish *N. notopterus* in one year period falls under four phases such as preparatory, pre- spawning, spawning and post-spawning. The important phases pre spawning and spawning phases are considered for the present studies.

The erythrocyte count in the fish during pre-spawning was 1.56 ± 0.03 M/mm^3 in male and 1.45 ± 0.03 M/mm^3 in females. The erythrocyte count is decreased in spawning compared to pre-spawning (1.44 ± 0.18 in males, 1.22 ± 3.73 in females). Haemoglobin levels also decreased during spawning, while haematocrit, MCV, MCHC levels are increased in spawning phase.

The blood parameters such as protein, cholesterol, urea, urea nitrogen and creatinine levels are increased during spawning compared to pre spawning phase. The glucose level found to be decreased during spawning as compared to pre-spawning in both male and female fishes (Table 1 & 2).

There is high positive correlation between BUN and glucose, haemoglobin, and haematocrit contents respectively in both the reproductive phases with mean 'r' values of 0.755, 0.795, 0.705 ($p < 0.05$) for BUN/glucose, BUN/Hb, and BUN/Hct respectively (Table 3 & 4) where as negative correlation shown between urea and BUN, glucose, haemoglobin, and haematocrit contents respectively in both the reproductive phases with mean 'r' values of -0.711, -0.725, -0.806, 0.715, ($p < 0.05$) urea/BUN, urea/glucose, urea/Hb, urea/Hct respectively (Table 3 & 4) indicating that as one variable increases, the other decreases.

High positive correlation was recorded between erythrocyte count,(RBC) haematocrit, and MCV levels in both the reproductive phases in fish *N. notopterus* with mean 'r' values for RBC/Hct = 0.737 and RBC/MCV = 0.755, $p < 0.05$ respectively, where as there is

Table 4: Intraspecies Haematological relationship in *Notopterus notopterus* (P<0.05) during Spawning phase

BUN	CHOL	CREA	GLU	HB	HCT	MCH	MCHC	MCV	PRO	RBC	UREA
CHOL	0.522	.									
CREA	0.507	0.249	.								
GLU	0.003	0.648	0.397								
HB	0.001	0.403	0.545	0.000							
HCT	0.021	0.291	0.060	0.098	0.066						
MCH	0.841	0.612	0.242	0.790	0.256	0.449					
MCHC	0.550	0.415	0.386	0.344	0.128	0.254	0.022				
MCV	0.289	0.906	0.513	0.417	0.779	0.904	0.055	0.427			
PRO	0.066	0.460	0.658	0.247	0.030	0.041	0.151	0.830	0.127		
RBC	0.010	0.474	0.056	0.076	0.131	0.007	0.048	0.732	0.022	0.702	
UREA	0.023	0.829	0.148	0.003	0.005	0.047	0.809	0.382	0.473	0.072	0.041

BUN = Blood urea nitrogen (mg/dl), CHOL = Cholesterol (mg/dl), CREA=Creatinine (mg/dl), GLU = Glucose (mg/dl), HB = Haemoglobin (g/dl), HCT = Haematocrite (%), MCH = Mean Corpuscular Haemoglobin (pg), MCV = Mean Corpuscular Volume (fl), MCHC = Mean Corpuscular Haemoglobin Concentration (%), PRO = Protein (g/dl), RBC = Erythrocyte count in million /mm3, Urea in (mg/dl)

Discussion

Fish reproduction is one of the factors seriously affecting the internal milieu of the organism. Therefore, a great attention has been paid to the study of haematological and biochemical indices during the reproductive period. The spawning phase falls during August – September after the full gonadal development from April–July (pre-spawning) in both male and female fish. Present work was undertaken to study the changes in the haematological levels of the fish *N. notopterus* during the two reproductive phases.

A change in haematological indices of tench (*Tinca tinca L*) during the reproduction period has been reported by Einszporn-Orecka (1970) and Svobodova *et al*. (2000). They reported that there is a drop in values of haematological indices particularly in erythrocyte count (RBC), haemoglobin content (Hb), and haematocrit value in the reproduction period of brood tench in lakes; an expressive decrease of values was found in females. Similar changes in haematological values (RBC, Hb, and Hct) were noticed after artificial reproduction in tench females and males by Svobodova *et al.*, 2001 also.

In *Salmo gairdneri*, *Cyprinus carpio* and *Salvelinus fontinalis*, Houston and De Wilde (1968) also reported that haematocrit values should be used as a general indicator of "hematological status" in routine examinations. They further found that there is correlation coefficient (r) of these variables was low, less than 0.5. The observations of the present investigation are in confirmation with the observations in *Scomberomorus maculates (* Pitombeira and Martins,1970), in *Tilapia zilli*, (Ezzat *et al.*, 1974); in *Oncorhynchus mykiss*, (Wells and Weber,1991) and in *Oncorhynchus mykiss* and *Dicentrarchus labrax* (Garcia *et al.*, 1992).

Many investigators also reported that fish deposits nutrients in their tissue (liver), these deposited foods were used during reproduction and for other activities (Nikolsky,1963). Nikolsky (1963) has also reported that peak values of glucose was noticed in male and during ovulation in females (spawning). Similar observation has been noticed in the present study. The serum glucose levels in both sexes of *N. notopterus* suggesting that the glucose could be used for reproductive process specially during spawning phase.

Kocaman *et al. (*2005) related difference in glucose concentration in the rainbow trout (*Oncorhynchus mykiss*) prior to the reproduction. Some authors also reported that serum glucose levels elevated until early spawning than rapid fall was observed in associated with reproduction. Contrary, a few investigators reports that the fish do not show differences in blood glucose concentration in females and males (Edsall, 1999; Bhatnagar and Saksena, 1989).

Concentration of total protein (TP) in blood was used as a basic index of condition and health status of fish (Mulcahy, 1971; Svobodova and Parova,1977; Hille, 1982; Jirasek *et al.*, 1993). In accordance with the finding reported above a significantly higher TP concentration during spawning was found by Svoboda *et al*. (2000) and Kocaman *et al.(* 2005). As evident from the literature the effect of sex on TP concentration in blood cannot be answered unequivocally. Often this effect is overlapped with other factors such as annual

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