Volume : 5 | Issue : 8 | August 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

Original Research Paper

Effect of Seasonal Variations on Differential and Total Leucocyte Value in *Ctenopharyngodon Idella*

Zoology

KEYWORDS : Seasons, Leucocytes, Haematological parameters and Ctenopharyngodon idella .

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ABSTRACT

The aim of this study was to determine the significant seasonal variations in the number of leucocytes in the blood of Ctenopharyngodon idella. Differential Leucocyte Count of a fish indicate the immunological and physiological healthy state. In the present study the DLC of grass carp in Lymphocyte shows that values were higher in post breeding season in the sexes. These values were again to the lower side in breeding season. Monocyte in the grass carp indicates that the mean values were in higher side in pre-breeding period and post –breeding period,the lower values were noted in the breeding period. Neutrophil of this carp indicates that the mean values were higher in the breeding season lowering in the post –breeding season and moderate in the pre-breeding season. Eosinophile studies also indicates that the mean values were higher in the breeding period gradually declining in the post – breeding period as well as in pre- breeding period. In the study of basophil it was found that mean values were higher in breeding period as 1.2 ± 0.07% in male fish. In the post-breeding period the values were recorded as 0.9 ± 0.02% and in the per- breeding period the values recorded as 0.9 ± 0.085% . Total count of leucocytes, differ from species to species. It is also related to sex; age, sexual difference, environment and season.

The leucocytes counts were affected by endogenous and exogenous factors such as breeding periods, water quality etc. The leucocytes value rice continuous by up to certain immune power, length, weight range, after which there occur a gradual fall.

INTRODUCTION

The studies on the haematology and blood bio-chemistry of different species of fishes are the great signification. It help in understanding the variation seen in the blood characteristics of fishes in selection of food and of Life.

The piscian-reptilian line , where in lymphocytes out – number the rest of leucocytes.Other line can be designated as amphibian- avian – mammalian line where in heterophils outnumber the rest of the leucocytes. There is no distinct difference in differential in differential count among the members of two sexes belonging to the same class group (Pai and Shanbhag , 1989).

The environmental factors such as temperature , pH , dissolved oxygen of water and quality and quantity of food are supposed to be main factors in the DLC variation (Smith et. al. 1952).The following five types of leucocytes with corresponding percentage given in this study were noted in C. catla : Thrombocytes (30) , Neutrophil (18) , lymphocytes (32) and Eosinophil (16). The percentage of basophile in C. catla is (4) which is much less than that of A. hexagonalepis but others failed to notice any basophil cells in their studies of the blood of the fresh- water Indian air- breathing fishes (Subba Rao and Behera, 1973).

The leucocyte value rice continuous by up to certain length, weight range, after which there occur a gradual fall. The effect of seasonal cycle was correlated to the total count of leucocyte .That the count was higher in summer. However,equivocal results were obtained in various teleosts. These studies showed that the count of leucocyte was more dependent on the sex,activity,diet,temperature and oxygen levels in the water (Bagchi and Ibrahim (1974),Agrawal, et.al.(1976) Dheer (1988).

It is due to the differences in the metabolic demands of the growing and maturing fish.The sexual difference in the number of leucocyte seem as to be a characteristics feature of some species of fishes. It appear s that the gonadeal activities and other associated endocrine factors have some bearing other associated endocrine factors have some bearing on this sexual difference(Alonso-Gomez, et.al. (2003).

In the present investigation of *Ctenopharyngodon idella* , haematological oveserved on the basis of seasonal variation and the number of Leucocytes(TLC & DLC) in relation to other blood contents, body weight, the influence of environmental factors and to sexual maturation and reproduction. *ctenopharyngodon idella* came to India from Hong-Kong. It's an economically valuable and culturally fresh water fish, its local name also Grass carp in India. This fish is also found in almost all the ponds and tanks so ever mostly vegetation are found because this is almost exclusively a vegetation are found because this is almost exclusively a vegetarian.

MATERIALS & METHODS

Study Area and Data collection - Live and healthy specimen of *Ctenopharygodon idell* were collected locally from water resources around Durg district throughout the year. The fishes were washed with 0.1% KmnO4 solution to avoid any fungal or dermal infection. They were narcotized by putting the cotton plug soaked with 90% alcohol over the gills. All these fishes after collection of the sample of blood in the field were brought to the laboratory in 10% formalin for their physical measurements and sexual identification (Conroy1972). The fishes for the present investigation were collected from the fresh water resources, as Kutela Bhata. Fish farm. Selud tank, Maroda tank, Surholi village tank, Khudmura, Deverbija, village tank, of Durg District in the vicinity of Durg district during the period of June to May.

Haematological analysis - Total leucocyte counts - The number of leucocytes contained in I liter of blood is called the total leucocytes or white blood cell count. It is usually expressed as the number of cells per cubic millimeter (μ 1). Counting of white blood cells of fish blood was done by improved neubauer rules counting chamber. The white cell were counted in the four corners of 1 square millimeter and in the central area on both sides of the counting chambers of the haemocytometer (10 square millimetes in all). The white cells were recognized by the refractile appearance and by the slight given to them by the stain contained in the diluting fluid.

TLC was made using an improved Neubauer haemocytometer. (Smith,et.al. 1952, Ezzat et.al. 1974 and Humason 1979).

Volume : 5 | Issue : 8 | August 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

Water analysis -D.O. of the water was determined by sodium azide modification of winkler's method (A.P.H.A, 1985). Determination of DO and Water temperature. Water temperature values were recorded by mercury bulb thermometer.

Determination of Gonadosomatic index:
G.S.I was calculated as- G.S.I= Gonad weight/Fish weight×100 .

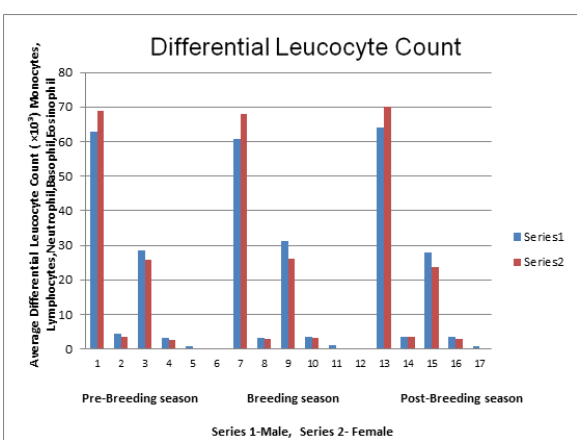
Statistical Analysis: Statistical analysis was performed with SPSS version 10.0 for windows (SPSS,1996). Data was presented as Mean±Standard deviation (S.D) of the mean and analyzed by one way analysis of variance.

Table : 1 Diferential Leucocyte Count (DLC) in male and female *Ctenophrygodom idell* (Valencinnes) during Pre – breeding period (PB), Breeding (B) and Post – breeding (POB) period showing seasonal variation.

S.N.	Types Of Leucocytes	Pre Breeding Season		Breeding Season		Post – Breeding Season	
		Male	Female	Male	Female	Male	Female
1.	Lymphocytes	62.8 ± 0.87	6801 ± 0.82	60.9 ± 1.43	68.0 ± 0.72	64.2 ± 0.64	70.0 ± 0.40
2.	Monocytes	4.5 ± 0.08	3.5 ± 0.13	3.2 ± 0.10	2.9 ± 0.08	3.5 ± 0.07	3.4 ± 0.09
3.	Neutrophils	28.6 ± 0.63	25.9 ± 0.47	31.1 ± 0.66	26.0 ± 0.78	27.9 ± 0.76	23.6 ± 0.72
4.	Eosimophils	3.2 ± 0.11	2.5 ± 0.11	3.6 ± 0.06	3.1 ± 0.07	3.5 ± 0.12	3.0 ± 0.09
5.	Basophils	0.9 ± 0.08	NIL	1.2 ± 0.07	NIL	0.9 ± 0.02	NIL

All Values Are Mean ± Standard Error.

For 8 Observation 4 Male And 4 Female On Each Month.



RESULTS

Differential Leucocyte Count : Differential leucocyte count of a fish indicate the immunological and physiological healthy state. In the present study the DLC of grass carp *Ctenopharyngodon idella* was done and following results was obtained.

Results of lymphocyte show that values were higher in post breeding season in the sexes. In male fish it was 64.2 ± 0.64% and in female fish it was 70.0 ± 040% .These values were found gradually decreasing in prebreeding season. they were found 62.8 ± 0.87% in male fish and 68.1 ± 0.82 % in female fish .These values were again to the lower side in breeding season ,the were 60.9 ± 1.43 % in male fish and 68.0 ± 0.72 % in the female fish.

Result of monocyte in the grass carp indicates that the mean val-use were in higher side in pre-breeding period and post – breeding period,the lower values were noted in the breeding period.In pre-breeding season in male fish the mean values were 4.5 ± 0.08% and 3.5 ± 0.13% in the female fish.During breeding season the male shows 3.2 ± 0.10% and female shows 2.9 ± 0.08 % .In the post –breeding period the values were 3.5 ± 0.07 % in the male fish ,and 3.4 ± 0.09 % in the female fish.

Results of neutrophil of this carp indicates that the mean values were higher in the breeding season lowering in the post – breeding season and moderate in the pre-breeding season. During breeding season the male fish imdicate 31.1 ± 0.66% and female fish shows 26.9 ± 0.78 % .During post- breeding season the mean values were found 27.9 ± 0.76% in male fish and 23.6 ± 0.72 % in the female fish. In the pre-breedisng season the mean values were found 28.6 ± 0.63 % in male fish and 25.9 ± 0.47 % in the female fish.

Eosinophile studies also indicates that the mean values were higher in the breeding period gradually declining in the post – breeding period as well as in pre- breeding periok. In the breeding season the values were found 3.6 ± 0.06 % in male fish and 3.1 ± 0.07 % in the female fish. During post- breeding period the mean values recorded as 3.5 ± 0.12 % in male fish and 3.0 ± 0.09 % in the female fish. In pre- breeding period the mean values were recorded as 3.2 ± 0.11 % in male fish and 2.5% ± 0.11% in the female fish .

In the study of basophil it was found that they were recorded in the male fish and in the female fish their presence was not recorded clearly.The mean values were higher in breeding period as 1.2 ± 0.07% in male fish. In the post-breeding period the values were recorded as 0.9 ± 0.02% and in the per- breeding period the values recorded as 0.9 ± 0.085% .

DISCUSSION

The documentation of literature represents the following discussion of DLC. The result indicates that birds and mammals have higher leucocytes counts than those for the other three groups of vertebrates. Which are taxonomically at lower level, Reptilian forms show the lowest leucocytes counts when compared with the values of other poikilothernms . Among homeotherms highest leucocytic counts are seen in Aves. Mammals have the leucocytic counts very closer to that of Aves (Pai and Shanbhag. 1989, Mishra (1993) and Gupta et. al. (1995).

Natarajan, G.M. (1981) observed in M.gulio the mean percentage of lymphocytes (45.9) , neutrophils (6.4) and basophils (0.3) to be higher than in Channa guchua (44.7,602,0.2).

In Clarias batrachus differential leucocyte counts were 4.41% (male) and 3.98% Large lymphocytes , 64.33 (male) and 63.93% (female) small lymphocytes, 28.52 % (male) and 29.48%(female) neutrophils, 2.31% (male) and 2.10% (female) eosinophils, and 0.43% (male) and 0.51% (female) monocytes . Differential count reveals that the precent distribution of lymphocytes and eosonophils is significantly higher in males vhile neutrophils and monocytes are significantly higher in female Claris batrachus.

Kumari et. al.(1989) observed in Cyprinus carpio the differential count neutrophils (50-60%) and eosinophils (2.0-5.0%) to be higher lymphocytes (37-47%) and basophils (0) were observed to be lower and monocytes (1.0-3.0%) were of comparable level with Labeo species.

In the control C.punctatus differential leucocyles were 10 ± 1.50% large lymphocytes 34 ± 1.63% small lymphocytes, 20 ±

0.69% neutrophils, 20 ± 0.78% basophils,12 ± 0.59% eosinophils and 4 ± 0.86% monocytes. In the control C. striatus differential leucocye counts were 10.7 ± 0.46% large lymphocytes,50 ± 0.16% basophils,3.2 ± 0.23% eosinophils. In the control G.gotyla gotyla differential counts were 15 ± 0.66% basophils, 15 ± 0.59% small lymphocytes, 17 ± 0.55 % neutrophils , 16 ± 0.69% basophils, 15 ± 0.59% eosinophils and 4 ± 0.76% monocytes (Thakur and Sahai 1994).

The variation observed in the DLC may be due to the physiological and ecological state of fishes. The environmental factors such as temperature , pH , dissolved oxygen of water and quality and quantity of food are supposed to be main factors in the DLC variation (Smith et. al. 1952)

LYMPHOCYTE

Lymphocytes of fishes has been discussed by few workers. These cells are immunocompetant cells according to Wintrobe (1967) . Robertson et.al. (1960), described the role of these lymphocyte the lymphocytes on their size, the large lymphocyte and small lymphocyte, In these two types of lymphocytes, small lymphocytes have been discussed as actively as actively migratory cell of non –phagocytic in nature.

MONOCYTE

In literature the accounts of monocyte in fishes have been discussed by few workers. So far I have gone through the literature, found that lymphocyte and monocyte are related to each other and both are related to other connective tissue cells.

Siddiqui et. al. (1970) and Subba Rao Behera (1973) did not find monocytes in catla ,Latla and Cirrhina. They also discussed that there in no significant difference in the percentage of these cells between immature and mature fishes or between the sexes . Mishra (1993) haave found 8 to 18% monocyte in Clarias batrachus .

Monocytes (13%) are irregular of slightly elongated in shape. The nuclei are eccentric ,large and made of loose charomatin fibrils and take purplish stain in cat fish (Rita rita) according to Pandey et.al. (1979)

NEUTROPHIL

Neutrophils are the most important of the leucocytes as they show the greatest sensitivity to change in the environment. Their characterization and identification in therefore of significance for assessing the changes in the physiological state of fishes.

Siddiqui et.al.(1970) reported 1.0 to 10.0% neutrophils in carps (Labeo,Catla and Cirrhina) but Subba Rao and Behera (1973) recorded 18.0% in Catla catla . They did not give separate values for males and females.Pandey et.al.(1979) observed decrease in neutrophils with increasing body weight in H.fossilis .None has so far reported lobed nucleus in neutrophils in any Indian fish. In Labeo,Catla and Cirrhina the neutrophil percentage is higher in mature fishes than in the immature ones,and at adult stage there is no significant difference in percentage in males and females .

EOSINOPHIL

Joshi (1988) and other workers have noted that eosinophils were scanty in number in various species of fishes and it has also been found .that they have never seen in all the species of fishes.

Vanvuren and Hattingh(1976) have discussed that eosinophils inactivate the histamine or histamine like toxic materials by their detoxifying property. Eosinophils are migratory but apparently not phagocytic in nature. They are more often found outside the vascular system than in the blood.

Pandey et.al.(1979) observed decrease in the percentage of eosinophils from liwer to higher weight groups in Hateropneustes fossilis Prasad and Banerjee (1982) reported no visible fidderence in the percentage of eosinophils between immature Catla and Labeo.

BASOPHIL

Various workers have discussed about the basophil as we go through the literature. It has been suggested that in normal blood, there is a very small number of basophils.No basophils were seen in any blood smear of red sea fish. In Indian water fish however basophils have been observed.

Rao and Behera (1973) studied the blood of the fresh water teleost Catla catla. They found basophil granulocytes in the blood smear of this fish but others failed to notice any basophil cell in their studies of the blood of some other fish. Thakur,et.al. (1994) have observed basophil granulocytes in the blood smear of Channa punctatus. Channa striatus and Garra gotyla. Nayak and Madhyastha (1982) have not basophils have been in their observation in Rasbora daniconius. Siddiqui et. al. (1970) reported the absence of basophils in the blood of Crirhina and Catla but Subba Rao Behera (1973) recorded 4.0% basophils in the blood of Catla catla. Prasad and Banerjee(1982) investigated that these cells are absent in immature Catla and Cirrhina and in mature female Labeo. The largest basophil is found in mature Cirrhina.

Mahajan and Dheer (1979), found 2.6 to 4% basophils in Channa punctatus. Catton (1951), has noted that the basophils are generally absent or rarely seen in blood films.

Table : 2 Total Leucocyte Counts (Thousand/Cubic Mm) in male and femal Ctenopharyngodon Idella (Valenciennes) during Pre – Breeding (Pb) , Breeding (B) and Post – Breeding (Pob) period showing seasonal variation.

S. NO.	Season	Months	Male		Female	
			Monthly Variation	Seasonal Variation	Monthly Variation	Seasonal Variation
1.	**Summer** (PB)	March	4.30 ± 0.08	5.80 ± 0.11 (4.20 – 7.10)	5.20 ± 0.09	6.32 ± 0.15 (5.00 – 7.50)
		April	5.90 ± 0.06		6.00 ± 0.08	
		May	6.00 ± 0.06		6.90 ± 0.17	
		June	7.00 ± 0.11		7.20 ± 0.12	
		July	6.20 ± 0.14		6.90 ± 0.15	
2.	Monsoon (B)	**August**	6.60 ± 0.33	6.90 ± 0.32 (6.10 – 8.10)	7.30 ± 0.19	7.55 ± 0.32 (6.40 – 8.50)
		September	6.80 ± 0.25		7.60 ± 0.14	
		October	8.00 ± 0.11		8.40 ± 0.22	
		November	7.80 ± 0.06	(7.10 ± 0.07) (6.30 – 8.00)	9.30 ± 0.16	8.17 ± 0.13 (7.00 – 9.40)
3.	Winter (POB)	**December**	7.20 ± 0.12		8.50 ± 0.15	
		January	6.90 ± 0.08		7.30 ± 0.13	
		February	6.50 ± 0.05		7.30 ± 0.16	

All Values Are Mean ± Standard Error.

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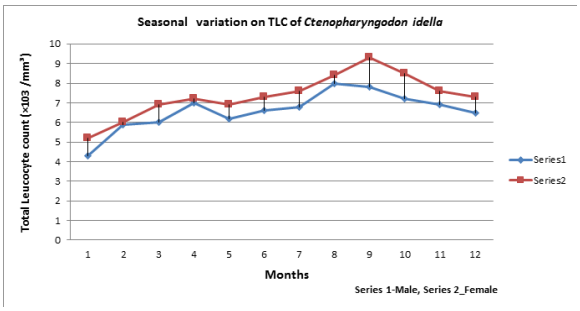

RESULT

The study of total leucocytes count was also done in 8 specimens with 4 males and 4 female fishes in each month . It was recorded that mean values were higher in post breeding season whereas the values were comparatively somewhat lowest in pre-breeding season .

The mean values of TLC of post-breeding period the values were recorded as 7.1 thousands per cubic mm in male fish with the seasonal variation 6.3 to 8.0 thousands per cubic mm with seasonal variation of 7.0 to 9.0 thousands per cubic mm.

In breeding period shows 6.9 thousands per cubic mm in male with their seasonal variation as 6.1 to 8.1 thousands per cubic mm . in female fish the values were 7.55 thousands per cubic mm with the seasonal variation of 6.4 to 8.5 thousands per cubic mm.

In the pre-breeding period , the mean values were found as 5.8 thousands per cubic mm in male fish with seasonal variation of 4.2 to 7.1 thousands per cubic mm .In female fish the values were 6.32 thousands per cubic mm , with seasonal variation of 5.0 to 7.5 thousands per cubic mm.

DISCUSSION

The documentation of literature represents the following discussion of TLC. Subha Rao and Behera (1973) observed the total number of WBC in C catla varied from 7.400 to 11,500/c.mm of blood with an average of 9,241 the male fishes have a higher count (9,816) than the females (8,660) .Siddiqui and Nseem (1978) studied the leucocyte count of Labeo rohit ranged from 3.800 to 10,000 mm^{-3}, with mean of 57 × 103 cells/mm3 of blood has been observed. Banerjee & Banerjee (1987) reported that the WBC number in Labeo rohita is significantly higher than in the males during these three periods. In this fish the WBC number is significantly higher during prebreeding and insignificantly higher during post-breeding periods than in the breeding period. This lower value during breeding period indicates that at los tempera –ture during winter months the haemopoietic loci for WBC production have increased activity. But this requires further study before a definite conclusion is drawn for fishes in general. Kumari et. al. (1989) reported that TCL of C. carpio was a 8.85 to 16.0 thousand/mm3, which is higher as compared to those of labeo species 3.92 to 7.30 tousand /mm3. Leucocytic counts in different sopecies are also variable ranging between 0.80 to 62.8 × 103 mm^3 and Bhatnagar and Saksena (1989)also observed in Clarias batrachus the leucocyte count was higher in female (15.44 × 103 mm^3) than in male (15.35 × 103 mm^3) . Ezzat et.al.(1974) who reported high leucocyte numbers in August . Banerjee (1982)in Channa punctatus also found the WBC when the RBC number increases in this fish. This may be accounted due to the fact that the erythropoietic center for RBC production is more active than leucopoietic centr.

Conclusion

In the present investigations, it can be concluded that, the effect of seasonal variation correlated to the total & differential count of leukocyte. That the count was higher in summer. These studies showed that the count of WBC was more dependent on the sex, activity, diet, temperature and oxygen levels in the water. The leucocytes value were affected by physiological and environmental factors such as diseases, breeding periods, water quality etc. This investigation may be helpful for monitoring the economic values, health status, breeding and related environmental factors.

AKNOWLEDGEMENT

Author thankful to Late Dr. B.N.Sharma Ex-Professor & Principal of Govt. College Vaishalinagar, Bhilai Durg, Hajjin K.Mansoori,Haji A.J. Mansoori Ex-Addi. Director,Department of Agriculture, Govt. Of M.P. and Dr. Avinash R. Nichat , Asstt. Professor,Govt. College Bhakhara for supervision of this work and encouragements and also Principal and Staff of Govt. V.Y.T. P.G. Autonomouse College Durg (CG) for providing necessary laboratory facility.

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