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Seasonal variation in leucocytes of mud *eel Amphipnous cuchia*

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Abstract : Morphology and number of the leucocyte or white blood cell reflects the health status and immunity of the fish as they are subject to change in accordance with the various environmental and physiological factors. Season comprises different phases or periods of the reproductive cycle greatly influence the number and morphology of leucocyte of *Amphipnous cuchia*. The number of all subtypes of leucocyte in *A.cuchia* exhibited a rhythmic variation throughout the year. The present research work is a comprehensive evaluation of number of white blood cells in relation to different phases of reproductive cycle correlating with the season.

Key Words: Lymphocyte, Monocyte Neutroohil, Eosinophil, Basophil Morphology Reproductive Cycle.

INTRODUCTION

Immunity of the fish is primarily concerned with the different types of leucocyte. Leucocyte or whiteblood cell is a good bioindicator which provide a better understanding of the physiological,pathological and behavioural state of the fish. The important environmental factors are season, temperature, gas and salinity etc. The major vital processes of fishes during reproductive cycle are tied to the most suitable season which ensures the maximum efficiency of constructive metabolic processes. All the types of leucocyte namely lymphocyte, monocyte neutrophil, eosinophil and basophil, provide a powerful defence mechanism against viral, bacterial and parasitic infections which occur during different season or phases of the reproductive cycle. Maturing and spawning phases are most crucial periods of the entire cycle and the fishes are most susceptible to the diseases and allergen . During these periods leucocytes are engaged in defensive and reparative function of the body by producing proper antibody.. The antibody invade antigen or allergen in several ways. There is a sharp increase or decrease in the types of the white blood cells and finally their number from the normal values.

The influence of the season or annual reproductive cycle in the fish haematology have been investigated by Robertson et al.,1961, Tugarina and Ryzhova;1970; Tandon Chandra,1976; JOSHI AND Tandon,1977, Khan l1977, Chanchal et al 1979 and Banerjee 1981,1982. Seasonal influence on white blood cell particularly has been reported by Ellis 1977 ,Mishra and Bhargava 1992 and others.

In the present study also a good relationship between white blood cell and reproductive cycles was observed . Individual form of leucocyte exhibited a varied picture also.

MATERIALAND METHOD

Healthy and matured fishes of both sexes of *Amphipnous cuchia* were collected from semiderelict swamps from surrounding areas with the help of local fisherman. Fishes were kept in a large aquarium sexwise in a group of ten fishes. Before putting them in aquarium they were dipped for a moment in potassium permanganate solution to get rid of microbial infection. Fishes were left for fortnight to acclimatize in the

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laboratory condition under natural photoperiod and temperature.

The acclimatized fishes of both sexes were sacrificed on each month of the year for evaluating annual fluctuation in differential count of leucocytes. Differential count was done through a well stained slide. A drop of immersion oil was kept on the blood film and the slide was examined through the microscope under the magnification of (i000x). 100 cells of leucocytes were counted properly by moving the slides between proximal and distal end along the narrow strip. From this the percentage of different subtypes of leucocytes were calculated.

RESULT AND DISCUSSION

From the table it is quite clear that in the blood of the *Amphipnous cuchia* the leucocytes exhibit variations in its morphology. Altogether five different morphological form of leucocytes were identified. They were Lymphocyte, Monocyte, Neutrophil, Eosinophil and Basophil respectively. The lymphocyts were of two sizes; the large lymphocytes and small lymphocytes. The number of lymphocytes was quite high throughout the year predominating over the rest of the subtypes in both sexes of the *A.cuchia*. The in male varied between 78-86 and in female 75-80.

Monocytes with typical kidney shaped or notched nucleus were the second highest in number among all the subtypes .The number of monocytes in male were 6-10 and in female 8-16 throughout the year. Neutrophils Eosinophil and Basophils exhibited a less percentage in both the sexes throughout the reproductive cycle of the fish. Their percentage were 2 to 4%, 3to 9% and 1 to 4% respectively. It was quite clear that male possesd higher number of lymphocytes neutrophils eosinophils and basophils than female fishes. However, the Monocytes were higher in female than male fishes.

Neutrophils were of large oval shape with multilobed eccentric nucleus and granular cytoplasm. Eosinophils were with bilobed nucleus and granular cytoplasm in few number. Basophils were comparatively smaller in size than other granulocytrs. It exhibited an oval or irregular shaped nucleus situated eccentrically in the cell. The cytoplasm of basophil was sparsely granular.

On reviewing the grand average values of differential count of leucocytes it was evident that male fishs possessed higher number of lymphocytes neutrophil eosinophils and basophils than the female. However the monocytes were higher in female fishes.

In fishes , leucocytes resemble the higher vertebrate leucocyte and are distinguished into granulocytes and agranulocytes which are further sub divided into lymphocytes, monocytes neutrophil eosinophil and basophil respectively. Lieb et al;1953 found only two types of lymphocytes e.g large and small in addition to monocyte, and neutrophil. Tugarina and Ryzhova (1970) and Ezzat et al (1974) also reported hetrogenicity in the leucocytes. In the present investigation all the five subtypes of leucocytes namely lymphocyte ,monocyte ,neutrophil eosinophil and basophil were found in blood of *A.cuchia*. Higher number of lymphocyte was reported by P RASAD (1980) IN Cirrhina, Labeo and Catla species. Singh and Banerjee (1992) have observed a higher percentage of lymphocytes even in marine fish *Pampus argentius*. Higher number of lymphocyte throughout the year was due to cope with the different environmental and physiological stress. The average number of lymphocytes during maturing and spawning periods in both sexes were to prevent the fishes from diseases and infections while gametogenesis and spawning start.

In female monocytes were higher to prevent the female from cellular infection as the female fishes were involved in much exhaustive task of oogenesis and spawning . Postspawning ,nonbreeding and preparatory phases were not much active phases as there were comparatively lower number of leucocytes. There was not much wear and tear during those periods. It may be presumed that the rate of leucopoiesis is decreased during these periods due to the low metabolic rate and unavailability of raw material. Seasonal variations in the number of all the subtypes of leucocyte was reported by several investigators like Gardnerand Yevich (1968) in *Fundulus fundulus*, Prasad (1991) in *Clarias batrachu*s and *Mastacembalus armatus.*.

In the present investigation these findings hold true in *Amphipnous cuchia*. Maturation and spawning of egg and sperm were regulated by different endocrines and hormones during these periods erythropoietin influence the rate of leucopoiesis also.

Table: Average differential leucocyte count in male and female *A.cuchia* during reproductive cycle.

Reproductive period	Month	Lymphocyte		Monocyte		Neutrophil		Eosinophil		Basophil	
		M	F	M	F	M	F	M	F	M	F
Preparatory phase	Feb	80.0	79.0	10.0	12.0	6.0	6.0	2.0	2.0	1.0	2.0
	March	82.0	80.0	0.0	8.0	7.0	7.0	3.0	3.0	2.0	2.0
	April	78.0	75.0	7.0	15.0	9.0	7.0	2.0	2.0	1.0	3.0
Maturing phase	May	81.0	79.0	9.0	10.0	5.0	6.0	2.0	2.0	3.0	2.0
	June	79.0	77.0	7.0	12.0	8.0	6.0	3.0	3.0	2.0	3.0
Spawning phase	July	84.0	76.0	7.0	16.0	6.0	4.0	2.0	2.0	2.0	0.0
	Aug	80.0	80.0	9.0	9.0	7.0	5.0	3.0	3.0	3.0	1.0
	Sep	80.0	75.0	7.0	10.0	8.0	9.0	3.0	3.0	3.0	3.0
Postspawning phase	Oct	81.0	78.0	10.0	12.0	4.0	5.0	3.0	3.0	2.0	2.0
	Nov	86.0	80.0	6.0	10.0	3.0	5.0	3.0	3.0	2.0	2.0
Non breeding phase	Dec	78.0	78.0	8.0	11.0	8.0	5.0	4.0	4.0	2.0	2.0
	Jan	80.0	75.0	8.0	14.0	6.0	8.0	3.0	2.0	1.0	3.0
mean		80.75	77.67	8.17	11.58	6.42	6.08	2.92	2.67	2.0	2.08
Standard error		±0.65	±0.56	±0.39	±0.69	±0.49	±0.40	±0.16	±0.18	± 0.20	±0.25

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