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HAEMATOLOGICAL STUDIES ON THE HEALTHY CATFISH *Clarias lazera*.

M.B. SHABANA AND S.E.M. KHADRE AND M.M. LOTFY

Department of Zoology, Faculty of Science,
Alexandria University - Alexandria, Egypt.

ABSTRACT

No significant difference due to sex was observed in the fish examined. However, seasonal variation was found to take place in RBC's, total leucocyte, thrombocytes and differential leucocyte counts. Fluctuations observed during different seasons were interpreted in the light of the seasonal activity of the haemopoietic organs during both prespawning and spawning seasons of the fish.

INTRODUCTION

Comparative haematological investigations in humans and other vertebrates have shown that blood plays an important role in reflecting the physiological condition. Therefore, it is essential to establish a normal range of haematological parameters for a particular species of animals. Although the haematology of two subtropical and African teleosts has been studied in some detail (Caxton-Martins, 1979), yet there is an urgent need for such information about most Egyptian fish and the catfish *Clarias lazera* in particular for its economic importance. This study is made to evaluate the basic blood characteristics of this fish.

MATERIAL AND METHODS

Samples of fish were collected monthly from Bab-El-Abid zone, Mariut, near Alexandria, Egytp. Sampling of blood was carried out after two days, keeping the fish in suitable continuously aerated aquaria filled with dechlorinated water.

Ten healthy fish were selected and examined monthly. Their body lengths ranged between 16 and 35 cm and weighed between 28 and 240 gm. each. The sex of each fish was determined prior to blood sampling which was made by severing the caudal peduncle. The blood was collected in heparinized vials to prevent rapid clottings.

Hendricks fluid (Hendricks, 1952) was used for erythrocyte count. Yokoyama's mixture (Yokoyama, 1960) was used for counting both total leucocytes and thrombocytes as described by McKnight (1966).

Blood smears, without heparin were made and stained by Panoptic method (Lucky, 1977). Morphological observation and differential leucocyte counts were made using stained smears.

Statistical analysis of the results was carried out according to Arkin and Colton (1963).

RESULTS

Erythrocyte Counts

Normal erythrocyte counts were made from 80 individuals during the rest period of the fish i.e. winter season, where no spawning occurs, so as to avoid any haematological disturbance. The fish were grouped on the basis of weight-length and sex as presented in Table 1. Fish were divided into five groups according to their weight.

The R.B.C's. count, fluctuates with increasing weight. Statistically, the correlation between the R.B.C's count and weight was found to be insignificant ($P > 0.05$). A similar result was also found as regards length and difference of sex (Table 1). It is also evident that; the mean RBC's count of the total five groups of fish arranged according to weight-length is more or less similar to that of fish arranged according to sex.

Table 1.

Erythrocyte counts of **Clarias lazera** arranged according to weight, length and sex.

Group	Weight (gm)	Length (m)	No. of Fish	Erythrocyte count ($\times 10^6$ mm^{-3}) Range	Mean
I	28- 68	16-22	10	1.814-2.436	2.292
II	69-107	22-26	9	1.476-2.568	2.088
III	108-147	23-29	7	1.764-2.616	2.240
IV	148-189	30-32	8	1.872-2.416	2.216
V	190-240	31-35	6	1.472-2.902	2.384
	Total		40	1.476-2.902	2.244
	Sex				
	Male		20	1.752-2.808	2.368
	Female		20	1.656-2.913	2.359
	Total		40	1.656-2.913	2.363

To study the seasonal variation in the R.B.Cs. count, blood analysis was made over five consecutive seasons, so as to complete one full year of experimentation and ensure overlaping of seasons. Thirty individuals of each sex were sampled during each season.i.e. ten samples from each sex monthly. The range and mean of the R.B.Cs counts for each month are summarized in Table 2.

It is evident that, the erythrocyte count undergoes cyclic changes throughout the year. It reaches its minimum value during spring season and its maximum during fall and winter.

Total Leucocyte Count

In the peripheral blood of *Clarias*, leucocytes are of four kinds, classified according to their form and affinity of dyes into neutrophils, eosinophils, basophils and lymphocytes. The first three are granular, whereas the last one is agranular. The lymphocytes are subdivided into small and large ones.

Table 2.

Erythrocyte counts of **Clarias lazera** during five seasons.

Season		Sex	Erythrocyte count ($X\ 10^6\ mm^{-3}$) Range	Mean
Spring	1986	M	1.035-2.885	1.750
		F	0.937-2.594	1.674
Summer	1986	M	1.405-2.445	2.021
		F	1.336-2.322	1.917
Fall	1986	M	1.730-3.320	2.323
		F	1.646-3.154	2.105
Winter	1986	M	1.650-3.085	2.277
		F	1.571-3.011	2.162
Spring	1987	M	1.275-2.673	1.930
		F	1.211-2.539	1.785

M = males, F = females.

The number of fish utilized of each sex is 30.

Clarias normal leucocyte counts were determined in 80 specimens during the rest period of the fish, the range and mean values are tabulated according to weight, length and sex in Table 3. As shown from this Table, the mean of leucocyte counts for individuals grouped according to weight-length is approximately similar to those reported for the same number of fish grouped according to sex. It is also evident that the total leucocyte count increases significantly ($P < 0.05$) as the fish increases in weight and length.

Seasonal variation pattern in the total leucocyte count is clearly different from that of the erythrocyte count. Table 4 shows that total leucocyte count decreases during the spring then increases to reach its maximum level during summer, thereafter decreases to reach a minimum value during fall, then increases again during winter.

Table 3.

Total leucocyte count of **Clarias lazera** arranged according to weight, length and sex.

Group	Weight (gm)	Length (cm)	No. of Fish	Leucocyte count ($X\ 10^3\ mm^{-3}$) Range	Mean
I	28- 68	16-22	10	25,780-33,460	28,504
II	69-107	22-26	9	36,830-43,640	37,800
III	108-147	23-29	7	31,640-51,080	45,487
IV	148-189	30-32	8	55,860-57,220	52,006
V	190-240	31-35	6	60,230-64,450	61,300
	Total		40	25,780-64,450	45,017
	Sex				
	Male		20	26,014-67,320	45,387
	Female		20	25,890-66,216	44,823
	Total		40	25,890-67,320	45,105

Table 4.

Total leucocyte count during five seasons.

Season		Sex	Total leucocyte cnt (x 10^3 mm^{-3}) Range	Mean
Spring	1986	M	36.250 - 44.606	41.72
		F	33.625 - 45.212	41.005
Summer	1986	M	38.840 - 56.520	51.160
		F	35.956 - 54.867	50.044
Fall	1986	M	27.460 - 35.230	33.340
		F	24.460 - 35.061	31.970
Winter	1986	M	37.160 - 50.780	46.220
		F	38.441 - 45.635	44.390
Spring	1987	M	36.660 - 45.20	41.980
		F	35.193 - 44.259	40.790

M = Males, F = Females.
The number of fish utilized from each sex is 30.

Differential Leucocyte Count

It seems that agranulocytes often constitute more than 40 % of the total number of leucocytes (Table 5). However, small lymphocytes contribute about 80 % of these agranulocytes. During spring, agranulocytes increase in number to reach a maximum level in summer. It was noticed that during this season, the number of large lymphocytes increases parallel to the increase of agranulocytes and also reach their highest level. This indicates that the increase observed in total agranulocytes is due to elevation of this type of lymphocytes, since small lymphocytes show a slight decrease than in spring. In fall, total agranulocytes decreases considerably due to the noticeable decline in large lymphocytes. During winter large lymphocytes show their minimum value. Accordingly, total agranulocytes reach their lowest level during this season.

However, two observations must be taken into consideration. First, neutrophils are often the most abundant type of granulocytes in *Clarias* blood. They range between 25.0 and 64.7 % during the four seasons of the year and reach their maximum level during fall and winter. This indicates that they are the major source that contributes to the elevation of total granulocytes during this season.

Table 5.

Ranges and means of differential leucocyte counts of **Clarias lazera** during five seasons.

Season	Sex	Neutrophil %	Eosinophil %	Basophil %	Total Granulocyte %	Small Lymphocyte %	Large Lymphocyte %	Total Agranulocytes %
Spring 1986	M	26.1-53.1 35.6	3.3-7.6 5.5	1.3-2.1 1.5	30.7-62.8 42.4	30.3-48.0 44.2	6.9-21.3 13.4	37.2-69.3 57.6
	F	28.6-61.2 40.3	2.1-5.3 3.9	0.6-1.1 0.9	31.3-66.6 45.1	27.6-45.[illegible] 41.3	3.3-24.7 15.6	35.9-69.8 54.9
Summer 1986	M	23.8-41.2 32.0	2.8-5.7 3.9	1.9-4.5 2.8	28.5-51.4 38.7	32.4-40.[illegible] 35.6	16.2-30.7 25.7	48.6-71.5 61.3
	F	20.9-43.6 30.3	2.5-6.4 4.1	1.4-2.5 1.9	24.8-52.5 36.3	31.8-47.7 39.4	15.7-27.9 [illegible]	47.5-75.2 63.7
Fall 1986	M	37.7-43.9 41.2	5.6-8.5 7.6	2.3-4.0 3.4	45.6-56.4 52.2	35.4-40.[illegible] 37.3	8.2-[illegible] 10.5	43.6-54.4 47.8
	F	39.6-46.4 43.5	4.8-9.1 6.8	2.6-5.2 3.7	47.0-60.7 54.0	33.1-41.8 36.8	[illegible]-12.2 9.2	39.3-54.0 46.0
Winter 1986	M	42.4-46.3 44.5	7.1-9.4 8.8	2.7-5.8 5.3	52.2-61.5 58.6	33.4-37.6 34.6	[illegible]-10.2 6.8	38.5-47.8 41.1
	F	39.8-44.5 41.8	6.7-8.6 8.2	2.3-5.5 4.9	48.8-58.6 56.6	34.3-39.7 35.7	7.[illegible]-11.5 7.7	41.4-51.2 43.4
Spring 1987	M	25.0-49.7 34.3	4.7-7.9 5.8	1.5-2.1 1.7	31.2-59.7 41.8	32.6-48.3 43.1	7.7-20.5 15.1	40.3-68.8 58.2
	F	29.4-64.7 40.6	3.0-4.8 4.7	0.8-1.4 1.1	32.2-70.9 46.4	22.9-44.7 39.3	6.2-23.1 14.3	29.1-67.8 53.6

M = male, F = female

The number of fish utilized from each sex is 30.

Second, although both eosinophils and basophils constitute comparative low ratios of the total granulocytes (Table 5) (eosinophils range between 2.5 and 9.4 % and basophils range between 1.3 and 5.8 %) yet they also reach their maximum values during winter.

Thrombocyte Count

Counts were made on 300 catfish taken form the beginning of spring 1985 to the end of spring 1986 (Table 6). Thrombocytes range form 5.978 to 28.914/mm^3 of circulating blood. As seen from the Table, the peak of thrombocyte count takes place during spring then decreases in summer and continues to decrease till it reaches its minimum value during fall. However, in winter, thrombocytes show a slight increase again.

Table 6.

Thrombocyte counts of **Clarias lazera** during five seasons.

			Thrombocyte count (x 10^3 mm^{-3})	
Season		Sex	Range	Mean
Spring	1986	M	14.305-27.538	20.286
		F	13.732-28.914	20.714
Summer	1986	M	12.506-21.565	16.802
		F	11.755-20.271	15.793
Fall	1986	M	9.610-19.223	13.495
		F	10.201-20.007	12.908
Winter	1986	M	6.317-20.295	15.623
		F	5.978-21.304	14.970
Spring	1987	M	13.794-28.627	21.025
		F	13.804-26.559	20.207

M = males, F = females.

The number of fish utilized from each sex is 30.

DISCUSSION

Data obtained from the present work regarding blood cell counts reveal that RBC's counts in **Clarias lazera** are within the ranges recorded previously for other fish species (Kawamoto, 1929; Gelineo, 1957; Sano, 1957; Snieszko, 1961; Schiffman and Fromn, 1959; McKnight, 1966 and Shabana, 1970). No significant correlation between the RBC's count and either weight or length of the fish was found. Sex difference is not exhibited, since both sexes have very similar means. This finding is in agreement with Katz (1951) and McKnight (1966) who reported no sexual variations in silver salmon and mountain whitefish were observed respectively.

As regards to seasonal variations in RBC's counts, cyclic annual changes were observed. They reach their minimum value during spring and maximum during fall and winter. It seems that higher RBC's counts recorded during fall and winter reflect increased erythropoietic activity during these periods in preparation to spawning which occurs in spring and summer times. In this respect, it is of interest to mention that during fall and winter the haemopoietic tissue of spleen in **Clarias** lazera is at the peak of its activity (Khadre, under publication). This observation suggests that spleen plays an important role in erythropoiesis of this fish.

The phenomenon of seasonal variation in RBC counts was also demonstrated in other fish species (Pavlov and Krolik, 1936; Young, 1949; Katz, 1951; Naumov, 1956; Gelineo, 1957; Sano, 1960 a & b; McKnight, 1966 and Shabana, 1970). However, the finding of Sindermann and Mairs, (1961) showed no seasonal variation in the blood of the alewives.

As regards to total leucocyte counts, the blood of **Clarias lazera** also shows no sexual difference, but there is evidence that the total leucocyte count increases as the fish increases in weight and length. In this respect, **Clarias lazera** resembles **Perca flavescens** (Yokoyama, 1960), **Prosopium williamsoni** (McKnight, 1966) and **Tilapia zillii** (Shabana, 1970) and (Ezzat et al., 1974). Seasonal variation in total leucocyte count was also noticed, and reached maximal level during summer and minimum value during fall. Seasonal variation in total leucycyte count was reported for many fish species (Katz, 1951; Naumov, 1956; Gelineo, 1957; Sano, 1960 a & b; McKnight, 1966; Shabana, 1970; and Ezzat et al., 1974).

Histological investigation of the kidney in Clarias lazera (khadre, under ppublication) reveals that its lymphomyeloid tissue, which is responsible for leucogenesis, is more active during spring. Therefore, the rate of leucopoesis esis increases in preparation to the next summer, when leucocytes reach their maximum level during the climax of spawning. During fall, on the other hand, this tissue appeares inactive (Khadre, under publication), therefore the leucopoietic rate decreases resulting in a lowest total leucocytic count.

It is a characteristic feature of differential leucocyte pattern of **Clarias lazera** that agranulocytes often constitute more than 40 % of the total number of leucocytes, although small lymphocytes constitute about 80 % of these agranulocytes. This finding agrees with those reported for other teleosts (Duthie, 1939; Yokoyama, 1960; Watsen et al., 1963 and Shabana, 1970).

During spring agranulocytes increase to reach their maximum level in summer. The elevation of agranulocytes was found to be due to the increase in large lymphocytes. In fall, however, agranulocytes decrease considerably probably due to the noticeable decline in large lymphocytes. Yokoyama (1960) reported that in spring, the lymphocytes constitute the greatest number of leucocyte in the perch. It is believed, that fluctuation in lymphocyte count noticed in the present work is a reflection to changes occurring in the activity of the immune system of **Clarias lazera**. Immunological data on teleost fish are still scarce, although, in the last decade much knowledge has been gained concerning the functional significance of leucocytes and related cells in the defense mechanism of vertebrates. Evidence now exists which subtantiates the immunocompetent nature of fish lymphocytes (Ellis, 1977). Nevertheless, conclusive evidence of the existence of T and B lymphocyte analogues is still lacking.

As regards the seasonal behaviour of granulocytes, two points of interest can be noticed. First, neutrophils are often the most abundant type in **Clarias lazera** blood, and reach their maximum concentration during winter. This observation indicates that neutrophils are the major source which contributes to the elevation of granulocytes during this season. The present finding is in agreement with observations of Weinreb (1963) on goldfish; Mcknight (1966) on mountain whitefish; Shabana (1970) on Tilapia zillii. Ferguson (1976) and Ellis (1976) on the plaice. Second, both eosinophils and basophils constitute lower proportions of the total granulocytes and they also reach their maximum values during winter. This observation is in complete accordance with that recorded for Tilapia zillii (Shabana, 1970).

During these experiments, it was noticed that thrombocytes count reaches its peak during spring, and its minimum value during fall, then increases again in winter. It was noticed that the lymphomyeloid tissue of the head kidney of **Clarias lazera** plays a major role in thrombopoiesis (Khadre, under publication). Therefore, it is responsible for the fluctuations that occur in thrombocyte activity. In spring, when this tissue is more active the rate of thrombopoiesis increases resulting in higher thrombocyte count. During fall, on the other hand, the inactivity of this haemopoietic tissue leads to lower thrombocyte count.

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