



**CITOMORPHOMETRIC EVALUATION OF KORAN (*SALMO LETNICA*)
ERYTHROCYTES UNDER NATURAL CONDITION**

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SYNOPSIS

This Paper presents the citomorphometric account of Koran erythrocytes under natural condition. Koran, an important endemic specie, grows in natural conditions in Ohrid lake. Ohrid Lake is a tectonic lake in south-east of Albania, distinguished for his ancientness and originality fauna. There are 10 endemic species of fish. *Salmo letnica* (Korani) (*Karaman 1929*) is considered as the most important and interesting endemic species, for its economic and commercial values.

A total of 65 individuals of fishes have been examined during the period 2006-2007. The blood has been drawn from caudal vein.

These indices has been evaluated with image technics. Determined the average values to area, length, breadth, perimeter, area of nucleus, interrelation N/C. The average values to these indices were: area 155.44 μm^2 , length 14.72 μm , breadth 10.13 μm , perimeter 40.80 μm , area of nucleus 21.48 μm^2 , interrelation N/C 0.23 μm .

The correlation coefficients beetwen erythrocytes indices have been also determined. The correlation beetwen area and perimeter were significance ($P > 0.000$). The correlation between area of citoplasm and area of nucleus were not significance ($P > 0.015$).

INTRODUCTION

Ohrid (Pogradec) Lake is a tectonic lake in the South-East of Albania. This lake is distinguished for its ancientness and the original fauna. It is considered like a living fossils museum. 10 of 17 autochthon fish of Ohrid Lake are endemic. There are two most important species, because of their scientific, ecologic and economic importance: koran (*Salmo letnica*) (Karaman 1929) and belushka (*Salmothymus ohridanus*) (Steindachner, 1892). Koran lives at depths of 60–80 m within the lake. Actually, these species are assigned like critically endangered ones and they are included in *IUCN Red List of Threatened Species*.

The studies about Koran and its forms present scientific interest for the researches of the Region. Up to now, the studies of Albanian researches have aimed to know the ecology, dietary regime, reproduction, etc. On the other hand, there are few studies of normal hematological and biochemistry indices of Koran by new methods such as electrophoresis, imagines techniques, PCR, etc.

Based on the reasons mentioned above, we undertook this study, the main aim of which was to determine normal values of erythrocyte and its indices, size of *Salmo letnica* in its natural conditions and the correlation between them. This study should help to clarify the taxonomic position of *Salmo letnica* forms, as well as.

MATERIAL AND METHODS

65 *Salmo letnica* adult fish, in natural conditions, were sampled. The samples, randomly picked up, were taken in summer and winter of 2006 - 2007, in Pogradec area, near Ohrid Lake (Lin, Pojskë, Piskupat, Zagorçan and Tushemisht). The blood was collected by puncture of caudal blood vessels, in the anaesthetized fish containing 62.5 mg/L solution of tricaine methanesulphonate (MS 222) [14]. The blood was collected using a heparinized syringe, and 6-8 ml of blood from each fish was placed into a separately labeled Vacutainer containing heparin. Each Vacutainer was vortexed immediately to insure mixing of the blood. Preparation of samples was performed in field conditions, immediately after blood collection and they were stored on ice, for not more than 24 hours.

There were calculated red blood cells count (RBCC), hematocrit (Htc), hemoglobin (Hb) and its fractions.

About 100 cells were measured in *Salmo Letnica*.

Erythrocytes and their nuclei were measured by means of an ocular micrometer.

The indices of erythrocytes has been evaluated with image technics. It was determined the average values to area, length, breadth, perimeter, area of nucleus, interrelation N/C. Hemoglobin concentration and cell counts were determined within 24 hours of sampling.

Hematocrit (Htc) level (cyanmethemoglobin method) was determined by using microhematocrit-heparinized capillary tubes and a microhematocrit centrifuge (14000

g/ 3 min) within 30 minutes [1]. In the current study, our aim was to describe and measure erythrocytes of *Salmo Letnica*.

Statistical Analysis

Means and standard deviations were determined for the haematological parameters. A one-way analysis of variance (ANOVA).

RESULTS AND THEIR DISCUSSION

1. Erythrocytes and their indices of *Salmo letnica*

Haematological parameters (RBCC) and size have been analysed in the blood of koran. These parameters and their statistically elaboration (mean values, minimal, and maximal bounders and standard deviation) are presented in Table 1 and Fig. 1 and 2. Values of erythrocyte indices of *Salmo letnica* are quite similar of those of other fish of *Salmonidae Family* [19, 13, 14, 7]. Confrontation of our data to the other authors shown there are similarities between species of *Salmonidae Family*, which they belong to [2; 13, 12].

Nr.	Hematological Indices		Value Interval	Mean \pm DS
1.	Erythrocytes	$\times 10^6/\text{mm}^3$	0.81-1.59	$1.11 \times 10^6 \pm 0.20$
2.	Hb	g/100mL	4.37-13.70	9.13 ± 1.9
3.	Hct	%	23-57	38.25 ± 6.2
4.	Area	μm^2	93.86-141.69	115.44 ± 9.25
5.	Length	μm	12.35-16.82	14.72 ± 0.89
6.	breadth	μm	8.88-12.22	10.13 ± 0.61
7.	perimeter	μm	36.60-44.86	40.80 ± 1.72
8.	Area of nucl.	μm^2	16.77-26.08	21.48 ± 1.83
9.	Interrel. N/C	μm	0.16-0.33	0.23 ± 0.03

Table 1. Erythrocytes and their indices of *Salmo letnica*

Variations of hematological data of *Salmo letnica*, compare with of the different authors, are linked not only with differences between species, but the influence of different ecological and biological factors of Ohrid Lake, as well. It is necessary to underline that our samples were of different age, size, sex and reproduction period. On the other hand, even though Koran is an endemic specie, its hematological data are approximately to the other species of *Salmonidae Family* [4, 5].

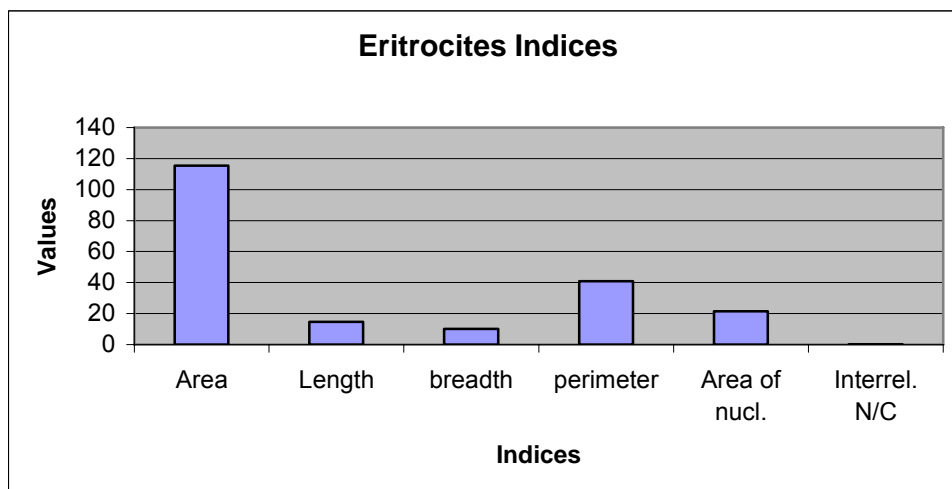


Fig 1. Erythrocytes and their indices of *Salmo letnica*

The studies on the blood of *Salmo letnica* described the structures, often comparing them with those of the other fish. The literature on hematology of fish blood is based on a few studies, with most concerned with European species (Saint Girons 1970).

Various authors have described the various circulating blood cells of different fish species (Ryerson 1949, Saint Girons 1970, Mateo et al. 1984, Alleman et al. 1999, Sevinç et al. 2000, Sevinç and Ugurta 2001, etc); some authors have studied seasonal (Haggag et al. 1966, etc) or sexual variations in the number of blood cells of different fish species. In our country, hematological studies have generally been conducted some economically important species. There are few hematological studies of the fish living in this country. Studies on our county are usually restricted to morphology and systematics.

The erythrocytes or red blood cells of *Salmo letnica* are nucleated, oval cells, and their nuclei are also oval and centrally located. The cytoplasm of mature erythrocytes appeared both light and dark pink and it is homogeneous under Wright's stain. The nuclei of mature erythrocytes are chromophilic (Figs. 2).

Because there were no significant differences between the erythrocyte sizes of female and male koran fish, the data from the females and males of individual species were pooled. The largest and widest erythrocytes were found in *Salmo Letnica*. The largest and widest nuclei were also found in *Salmo Letnica*. Erythrocytes function in the transport of oxygen and, to a much lesser degree, carbon dioxide. Molecular oxygen is carried on molecules of haemoglobin in the cells.

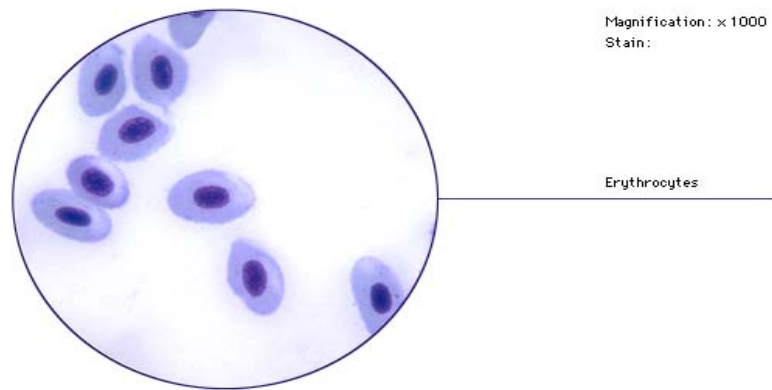


Fig. 2 Erythrocytes

There were significant differences between the area and perimeter of erythrocytes ($P < 0.000$). The other authors presented different results: there were no significant differences between the erythrocyte sizes (I. H. Ugurtash, 173-178, 2003). Interrelation area between N/C also was significant ($P < 0.000$).

The surface of the cell and its perimeter show a positive correlation, (5.09), and changes in the surface of the cell are accompanied parallelly with changes in its perimeter at the level of -92.3 units. This can be calculated using following formula $S = - 92.3 + 5.09$

Another standard which shows the correlation of these two variables is the R^2 value, which shows its correlation coefficient. The value of the correlation coefficient R^2 is 90 %, which is considered as excellent coefficient and statistically significant. This may be noticed observing the following figure.

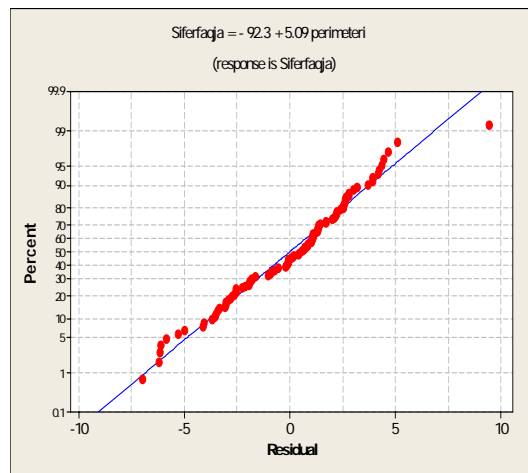


Fig. 3 Relationship between surface and perimeter erythrocytes

2. Relationship between erythrocytes and Haematocrite

Erythrocytes and haematokrite have a positive correlation and their value is 0.00392, which means the every change of the erythrocytes results with changes of the value of

haematokrite. This can be calculated as follows: Erythrocytes = $0.961 + 0.00392$ haematokrite. Another standard which shows the correlation of these two variables is the R^2 value, which shows its correlation coefficient.

In this case the value of the correlation coefficient R^2 is 1.6 %, which is considered as a weak model and statistically insignificant. This is shown in the following figure.

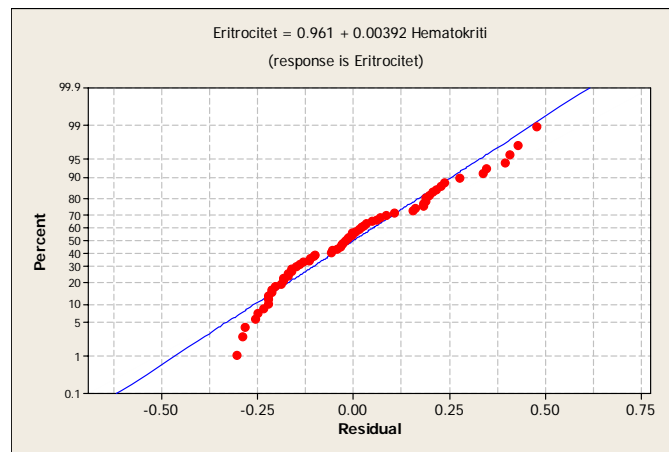


Fig. 4 Relationship between erythrocytes and haematocrit

Erythrocytes and haemoglobine have a positive correlation and their value is 0.0071, which means the every change of the erythrocytes results with changes of the value of haemoglobine at the level of 1,05 units. This can be calculated as follows: Erythrocytes = $1.05 + 0.0071$ haemoglobine. The value of R^2 is 0,5, which is considered as a weak model and statistically insignificant. This can be observed in the following figure.

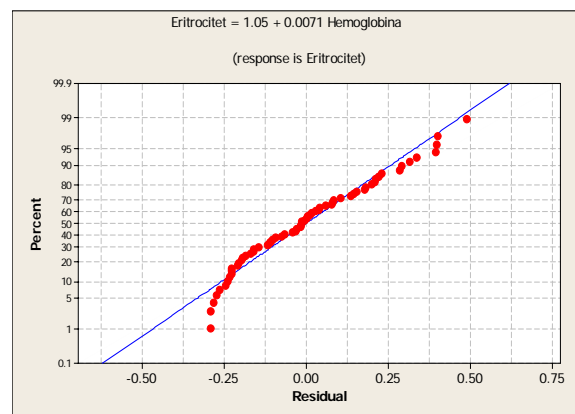


Fig. 5 Relationship between Eritrocite and Haemoglobine

The surface of the erythrocytes and the relation N/C have a negative correlation -186, which means that the surface of the erythrocyte and the relation N/C have a negative correlation and for every change in units of the erythrocytes results with

changes in the relationship N/C at the level of 158 units. This can be calculated using the following formula: Surface = 158 – 186 N/C.

In this case the value of the correlation coefficient R^2 is 32.2 % 0.5 , which is considered as a weak model and statistically insignificant. This can be observed in the following figure.

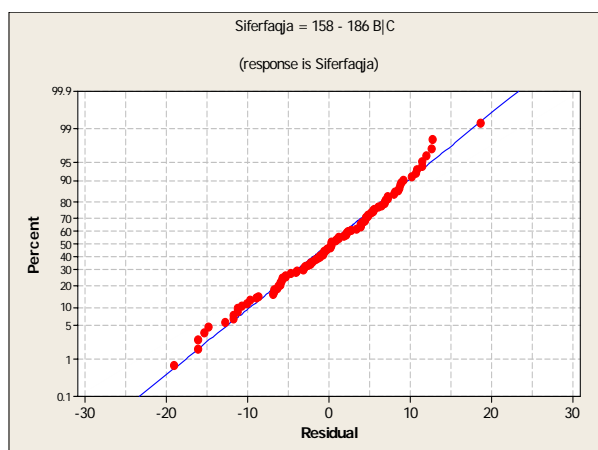


Fig. 6. Relationship between surface and N/C

CONCLUSION

- Values of erythrocyte indices of *Salmo letnica* are quite similar of those of other fish of Salmonidae Family, although it is an endemic specie of Ohrid Lake.

- Erythrocyte indices of *Salmo letnica* forms, in natural conditions are: RBCc $1.11 \times 10^6/\text{mm}^3 \pm 0.20$; Htc 38.26 ± 10.45 %, Hb $9.33 \text{ g/dL}^{-1} \pm 2.4$; Area 38.25 ± 6.2 ; Length 14.72 ± 0.89 ; Breadth 10.13 ± 0.61 ; Perimeter 40.80 ± 1.72 ; Area of nucleus 21.48 ± 1.83 ; Interrelation N/C 0.23 ± 0.03 .

- There were significant differences between the area and perimeter of erythrocytes ($P < 0.000$); also Interrelation Area between N/C also were Significant ($P < 0.000$).

- Correlation Between Erythrocytes and Hct were no significant ($P > 0.309$); correlation between erythrocyte and Hb were no significant ($P > 0.578$).

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