Journal of Research in Ecology

An International Scientific Research Journal

ISSN No: Print: 2319 –1546; Online: 2319– 1554

Original Research

Study the blood indicators for common carp fish *Cyprinus carpio* L. in the Euphrates river in the section passing through the city of Samawah

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ABSTRACT:

The blood indicators for the common carp (*Cyprinus carpio* L) was estimated from the Euphrates river in the section passing through the Samawah area in the Al-Khader region at different ages, weights, lengths, gender and temperature differences during the study period (five months). If groups of fish were selected every month. A group of three fish were selected by weight. Group A was with a mean weight of 140 ±4 g, group B was with 450±4 g and group C was with 800 ± 4 g with length of 26± 2 cm, 40±2 cm and 60±2 cm for groups A, B and C respectively. The results showed a difference in the measurements of the blood with the increase in age, height and weight off the fish groups. It reached high in the group C (0.70±0.11 cell×10^6 mm^3, 207±3.6 cell×10^3 mm^3, 42±2.8%, 12.6±0.4 g/dl, 167±1.98 mg/dl) for RBC, WBC, PCV, Hb and glucose respectively, and there showed no differences in the blood parameters within the same group. However, differences were observed between the same sex groups ($P<0.05$) for blood standards between coefficients at different temperatures during the months of the study.

Keywords:
Blood measurements, Common carp, Euphrates river.

Article Citation:
Miriam J. Mohammed, Taha YF. Al-khafaji and Dhellal M. Halbos
Study the blood indicators for common carp fish *Cyprinus carpio* L. in the Euphrates river in the section passing through the city of Samawah
Journal of Research in Ecology (2018) 6(2): 1932-1939

Dates:
Received: 01 July 2018 **Accepted:** 13 Aug 2018 **Published:** 17 Sep 2018

Web Address:
http://ecologyresearch.info/documents/EC0595.pdf

Journal of Research in Ecology

INTRODUCTION

Blood volume in fish is generally smaller than that of other vertebrates, as it ranges from 2-4 ml/100g in the bony fish (Osteichthyes) and is different in cartilage fish (Chondrichthyes) 6-8 ml/100g (AL-Daham, 1990). Blood cells in fish are often formed in the spleen in most of the fish and in some of them at the head of the kidney as especially, white blood cells in most of the bony fish. Blood consists of fish from plasma and blood cells, which include and blood cells Red Blood Cells (RBCs), or erythrocytes, White Blood Cells (WBCs) or leukocytes. Red blood cells contain no nucleus, the length of the cell in the bony fish is 12-14 microns and in the cartilage fish, the length is 20-27 microns. The number is about 101 million cells/cm^3. The white blood cells are not large in number of than the red cells in the blood, it was 150,000 cell/mm^3 in most species of fish and may be variable within the same species (Abdel-Razzaq and Mohsen, 1986).

White blood cells included four cell types (granulocytes, thrombocytes, lymphocytes and monocytes), the latter being the most numerous of the rest of the white blood cells in many marine fish, accounting for half of the total number, while the granulocytes has three types in general. The most common types of fish was (equivalent cells, acids cells, basic cells), the granules bearing cells were phagocytes, which are common to address disease and increase their numbers, especially when infected with bacteria (Binod *et al*., 2014). Lymphocytes form 15% of white blood cells in the common carp fish *Cyprinus carpio* L. and rainbow trout. The blood also transports many organic and organic substances such as hormones, vitamins and blood plasma proteins (2-6 mg/100 ml). These proteins contain two forms of alpha globulin and two forms of beta, gamma globulin, as well as albumin. These substances are used in the immune response, in pH changes, and in the regulation of important osmotic pressure in the movement of water through the walls of blood vessels (Abdel-Razzaq and Mohsen, 1986). The varies of concentration depending on the environment and fish species and in the blood of the bony fish is less than 200 mg of freshwater fish to more than 400 mg of marine fish. Both sodium and magnesium, as well as urea and free amino acids, have a significant effect on the concentration of blood osmosis for fish (Baghizadeh and Khara, 2015). Most of the food in the world was supplied by fish sources, so it was necessary to preserve fish life and ensure its health and spread in aquatic environments (Tripathy *et al*., 2002).

The blood measurements are one of the most important measures to maintain fish health. Celik (2004) pointed out that the measurement of blood parameters for all fish depended on age and detected physiological changes occurring within the fish bodies (Satheeshkumar *et al*., 2011) as well as early detection of any disease (Kori-Siakpere *et al*., 2005), within the species (Anthony *et al*., 2010) by age (Jamalzadeh and Ghomi, 2009) and gender (Gabriel *et al*., 2011). Indicators were based on the hormonal treatment of fish, espe-

Table 1. Measurements of ecological characteristics of water in the Euphrates river at the city of Samawah (Mean ± SD)

S. No	Measurements	Study period					
		February	January	December	November	October	September
1	Temperature °C	20^{b}	19^{c}	18^{c}	21^{b}	23^{a}	24^{a}
2	Concentration of dissolved oxygen (mg/l)	11.5 ± 1.14^{a}	10.12 ± 1.12^{b}	9.6 ± 0.92^{c}	9.4 ± 0.90^{c}	10.2 ± 0.83^{b}	10 ± 0.08^{b}
3	pH	7.8 ± 0.30^{a}	8 ± 0.41^{a}	7.6 ± 0.33^{a}	7.2 ± 0.30^{b}	7.0 ± 0.22^{b}	6.8 ± 0.20^{c}
4	Salinity (g/l)	1.12 ± 0.07^{b}	1.10 ± 0.08^{b}	1.20 ± 0.14^{b}	1.22 ± 0.83^{a}	1.30 ± 0.40^{a}	1.28 ± 0.42^{a}

*Different characters indicate significant differences ($P<0.05$) between the months in the same row.

Table 2. RBC and WBC count during the months of study in *Cyprinus carpio* L. (Mean±SD).

S. No	Study months	Blood parameters for fish group					
		RBC			WBC		
		A	B	C	A	B	C
1	September	$120^{C}\pm4.16^{c}$	$143^{C}\pm4.66^{b}$	$164^{AB}\pm4.48^{a}$	$0.24^{C}\pm0.38^{c}$	$0.37^{AB}\pm0.29^{a}$	$0.47^{C}\pm0.55^{b}$
2	October	$133^{B}\pm3.52^{b}$	$151^{C}\pm7.42^{ab}$	$168^{AB}\pm6.96^{a}$	$0.26^{C}\pm0.67^{c}$	$0.40^{B}\pm0.13^{b}$	$0.56^{B}\pm0.12^{a}$
3	November	$138^{A}\pm6.56^{b}$	$164^{A}\pm3.84^{a}$	$176^{A}\pm6.86^{a}$	$0.42^{A}\pm0.54^{a}$	$0.53^{A}\pm0.49^{a}$	$0.66^{AB}\pm0.12^{a}$
4	December	$138^{AB}\pm7.31^{b}$	$168^{A}\pm4.05^{a}$	$173^{A}\pm3.52^{a}$	$0.35^{B}\pm0.75^{b}$	$0.48^{A}\pm0.78^{a}$	$0.71^{A}\pm0.41^{a}$
5	January	$135^{A}\pm2.40^{c}$	$160^{AB}\pm2.60^{b}$	$171^{A}\pm3.84^{a}$	$0.32^{B}\pm0.23^{b}$	$0.44^{B}\pm0.65^{b}$	$0.51^{B}\pm0.75^{b}$
6	February	$144^{A}\pm2.81^{b}$	$152^{AB}\pm2.02^{b}$	$160^{B}\pm3.21^{b}$	$0.35^{B}\pm0.36^{b}$	$0.38^{AB}\pm0.21^{b}$	$0.48^{C}\pm0.40^{c}$

*The different small letters indicate significant differences (P<0.05) between the coefficients. *Different large letters indicate significant differences (P<0.05) between the totals for the same column.

cially during breeding (Baghizadeh and Khara, 2015), as well as the behaviour of the feeding fish (Satheenkumar *et al.,* 2011). Many researchers focused on the health of fish species as an evidence for fish assessment (Biond *et al*., 2008). They noted that blood indicators are influenced by sex and seasonal variations and the some environmental factors. It showed an impact on the blood standards for fish as an effective indicator of physiological and pathological changes in fish (Orun and Manelyazlak, 2003). Blood indicators were affected by several factors, including disease management (Svobodova *et al*., 2006; Collins *et al*., 2016) and stress (Grami *et al.,* 2004). In spite of the blood measurements for fish in Iraq was little, the current study aimed to measure the various blood parameters of common carp fish (*C. carpoi* L.) per month according to their age, weight, length and gender in the section passing from the Euphrates river in the city of Samawah.

MATERIALS AND METHODS

This work was conducted in the fish lab of the Faculty of Agriculture, Al-Muthanna University was conducted for the period from 15/9 until 15/02/2018. A different weight of common carp fish (*Cyprinus carpio*L) was selected. The fish were collected from a fish farm in Al-Khader region in Al-Muthanna Governorate by 25 l plastic bags. After saturation of water in the bags with sufficient compressed oxygen gas, ice sheets were placed inside the bags to reduce the temperature of transport water and reduced dissolved oxygen consumption during transport (AL-Daham, 1990). The fish were then placed in 35 x 70 x 30 cm water tanks containing 40 l of water and oxygenated the aquarium water during the working period until the fish were taken and meas-

Table 3. Hemoglubin and PCV during the study period in *Cyprinus carpio* L. (Mean ± SD)

S. No	Study period	Blood parameters for fish group					
		Hb (g/dl)			PCV (%)		
		A	B	C	A	B	C
1	September	$20^{C}\pm1.26^{c}$	$28^{B}\pm1.15^{b}$	$33^{B}\pm1.45^{b}$	$5.7^{C}\pm0.27^{c}$	$7.7^{C}\pm0.26^{b}$	$10.2^{AB}\pm0.20^{a}$
2	October	$25^{B}\pm1.76^{b}$	$31^{AB}\pm0.06^{ab}$	$34^{B}\pm2.02^{a}$	$8.2^{B}\pm1.09^{b}$	$10.0^{C}\pm0.41^{ab}$	$10.3^{AB}\pm0.54^{a}$
3	November	$28^{A}\pm1.15^{b}$	$31^{A}\pm0.09^{ab}$	$34^{B}\pm1.20^{b}$	$9.3^{A}\pm0.70^{b}$	$10.5^{A}\pm0.73^{a}$	$10.8^{A}\pm0.58^{a}$
4	December	$24^{B}\pm2.33^{b}$	$33^{A}\pm1.76^{a}$	$35^{A}\pm1.76^{a}$	$8.2^{AB}\pm0.43^{b}$	$9.2^{A}\pm0.58^{a}$	$10.2^{A}\pm0.58^{a}$
5	January	$20^{C}\pm0.37^{c}$	$30^{A}\pm1.30^{ab}$	$32^{B}\pm1.45^{b}$	$7.8^{A}\pm0.14^{c}$	$9.0^{AB}\pm0.51^{b}$	$10.4^{A}\pm0.17^{a}$
6	February	$21^{C}\pm1.45^{c}$	$28^{B}\pm1.15^{c}$	$31^{B}\pm1.10^{b}$	$7.0^{A}\pm0.24^{b}$	$8.4^{AB}\pm0.35^{b}$	$9.0^{B}\pm0.14^{b}$

* Different small letters indicate significant differences (P<0.05) between the coefficients and different large letters indicate significant differences (P<0.05) between the totals for the same column.

urements were made (Janncey and Ross, 1982). Each group consists of three fish. Then the fish were divided into three groups. The first group was called group A and the weights ranged from 140±4 g and the second group B. Their average weight was 450 ± 4 g and the third group C weighed 800±4 g, and their lengths were 26±2 cm, 46±2 cm, 60±2 for groups C, B, and A respectively. In addition, each species of fish was recorded for each group and the number of scales were taken into consideration for the estimation of the age. Four to five scales were taken from each fish in each group, cleaned well, washed with water and then soaked in sodium hydroxide solution (5 g/100 ml water), distilled for 24 h and in the next day, it was washed well, cleaned and placed between two slides, connected well and write down all the information of the fish and its collection to be seen under the microscope to estimate the age. Hematological tests were performed on the fish. The blood from the caudal vein behind the fin is extracted using 5 cm^3 plastic syringes and the following tests were performed (External laboratory).

Measurement of hemoglobin concentration

Drabkins reagent was used after placing it the blood in sterile test tubes and withdraw the blood from the experimental fish of through the sign (20) mediated by the Sahli pipette and mix well to get 251 dilution factor and leave for 5min to allow the conversion of hemoglobin into cyanomethaclubin and then put in the centrifuge at 2500 cycles/minute for 5 min. The results were recorded using a spectrophotometer after washing the tubes with distilled water. The device was then filtered by a Drakeniz Detector along a 540 nm wave length. The normal hemoglobin reading was recorded and then the original model was read (g /dl). Measurement of the percentage of the Packed Cells Volume (PCV %) were done following the method of Blaxhall and Daislly (1937).

Injection of blood from the venous vein was done using a plastic syringe (syringe) after moisturizing with heparin. The volume of the blood drawn was between 0.2 - 0.4 mL (blood mixture with anticoagulant) and then put the mixture on a slide, used capillary tubes with 75 mm length and 1.1 - 1.2 diameter. The blood was withdrawn and sealed from one end with the artificial clay and then centrifuged for 5 min. Microhematocrit centrifugation was done at the rate of 1500 cycles per minute and read the results Mediated by a special ruler in the microhematocrit reader. The results were expressed (%) representing the red blood cells per 100 cm^3 (Blaxhall and Daislly, 1973).

Statistical analysis

The data were statistically analyzed according to one way ANOVA using the Complete Randomized Design (CRD) program. To test the differences between the averages, Duncan multiple range test (Duncan 1955), was also used in the (Statistic Analysis System) (SAS,1992).

RESULTS AND DISCUSSION

Tables 1, 2 , 3 and 4 show the environmental measurements in the Euphrates river in the section passing through the city of Samawah at Al-Khader region. Differences in the temperature are observed during the months of the study. The highest reachs at the September (24°C) and the lowest at December (18°C) (Imsland

Table 3. Glucose during the months of study of the common carp fish *Cyprinus carpio* L. (Mean ± SD)

S. No	Study months	Blood parameters for fish group		
		Glucose (mg/dl)		
		A	B	C
1	September	$118^{B}\pm1.26^{b}$	$132^{B}\pm1.15^{a}$	$133^{C}\pm1.45^{c}$
2	October	$122^{AB}\pm1.76^{b}$	$133^{AB}\pm0.06^{a}$	$136^{B}\pm2.02^{b}$
3	November	$122^{AB}\pm1.15^{b}$	$134^{A}\pm0.09^{ab}$	$137^{B}\pm1.20^{a}$
4	December	$126^{A}\pm2.33^{a}$	$136^{A}\pm1.76^{ab}$	$143^{A}\pm1.76^{a}$
5	January	$123^{AB}\pm0.37^{b}$	$133^{AB}\pm1.30^{b}$	$140^{A}\pm1.45^{a}$
6	February	$121^{B}\pm1.45^{b}$	$128^{B}\pm1.15^{a}$	$130^{C}\pm1.10^{c}$

* Different small letters indicate significant differences (P<0.05) between the coefficients and different large letters indicate significant differences (P<0.05) between the totals for the same column.

Table 5. Blood parameters for groups (ages, lengths and weights) during the months of study of common carp fish *Cyprinus carpio* L. (Mean ± SD)

S. No	Fish group	RBC Cell×10^6mm^3	WBC Cell×10^3mm^3	PCV %	Hb g/dl	Glucose Mg/dl
1	**A** Less one year (1-), Length (26cm), Weight (140g)	0.38±0.05^c	165±3.8^b	30±0.23^c	9.3±0.70^b	149±0.05^b
2	**B** More than one year (1+), Length (40cm), Weight (450g)	0.44±0.07^b	191±4.5^a	37±1.2^b	11.3±0.73^a	158±2.45^{ab}
3	**C** Two years and more than (2+), Length (60cm), Weight (800g)	0.70±0.11^a	207±3.6^a	42±1.2^a	12.6±0.40^a	167±1.98^a

*Different letters indicate significant differences (P<0.05) between the groups within the same column.

et al., 2008). The decrease in oxygen concentration, pH and salinity is noted with different months. In November, the oxygen concentration was (9.4 ± 0.90 mg / L) and pH was 6.8± 0.20 while the concentration of oxygen and pH are inversely proportional to the temperature. Salinity was highest in the month of October, it reached (1.30±0.40 g/L). These differences are affected by different temperatures during the months of the study.

Differences in the blood measurements between groups A, B and C were also affected during the months of study, as well as between the same groups during the month. This indicated the effect of these blood measurements on different temperature during the studied months increased during the November for the three groups and for group A (0.42±0.54), (138±6.56), (28±1.15%), (9.3±0.70 g/dl), for WBC, RBC, Hb and PCV and noticed glucose increase in A group in the December which reached 126±2.33 mg/dl as well as an increase of C and B groups for the same month. While other blood measurements aggregated C, B in November as the WBC 0.66±0.12 cell×10^6mm^3 and the RBC of B group in December was reached 168±4.05 cell×10^3mm^3, while the C group in November reached 176±6.86 cell×10^3 mm^3 to RBC, Hb was varied For three group was a range of 22±1.15 g/dl in November and the B group was 33±1.76 g/dl. The level of PCV was the highest for the three groups in November (9.3±0.70, 10.5±0.73 and 10.8±0.58)% for A, B and C respectively, while the highest levels of glucose were observed in December for the three groups A, B and C as it reached 126 ±2.33, 136±1.76, 143 ±1.76 mg/dl indicated the variation in blood measurements by months of a year and different temperatures, as it increases in the cold months and decreases during the warm months. This is in contrast to Binod *et al*. (2014) who measured the *Catla catla* fish during the various seasons where RBC and Hb, increased during the summer (1.33-1.177 ×10^6 cells mm^3) and decreased in winter (5.5- 7.7 mg/dl). These differences may be due to the changes in the atmospheric temperature that affect the water temperatures Orun and Manelyazlak (2003)

Table 6. The blood parameters during the study period for *Cyprinus carpio* L. (Mean ± SD).

S. No	Fish group	Sex	RBC cell×10^6mm^3	WBC cell×10^3mm^3	PCV %	Hb (g/dl)	Glucose (mg/dl)
1	A	Male	0.32^C±0.05^a	160^B±36^a	30^C±0.8^a	9.3^B±0.70^a	149^C±2.40^a
		Female	0.36^C±0.08^a	156^B±3.3^a	28^C±1.1^a	9.0^B±0.60^a	146^C±1.95^a
2	B	Male	0.50^C±0.13^a	181^A±3.2^a	37^B±1.2^a	11.3^{AB}±0.73^a	158^C±2.45^a
		Female	0.46^C±0.05^a	187^A±3.5^a	35^B±1.3^a	11.0^{AB}±0.75^a	156^B±2.50^a
3	C	Male	0.70^C±0.11^a	195^C±4.6^a	42^A±1.2^a	12.6^A±0.40^a	167^A±1.98^a
		Female	0.66^C±0.13^a	191^C±4.5^a	39^A±1.3^a	12.0^A±0.41^a	163^A±1.96^a

*Different large letters indicate significant differences (P<0.05) for the same sex within the groups

reported that blood measurements were affected by water temperature and seasons.

Table 5 shows the difference in the parameters of the blood and the age, weight and length of the fish. As the age increases, the length and weight increases, the fish parameters were also increased. The highest ratio was found in C group at the age of two years with a weight of 800 g and length 60 cm and 0.70±0.11 cell×10^6mm^3, 207±3.6cell×10^3mm^3, 42±1.2%, 12.6±0.4 g/dl, 167±1.98 mg/dl, for RBC, WBC, PCV, Hb and glucose respectively. RBC, WBC, PCV, Hb, and glucose respectively. There results were in agreement with Baghizadeh and Khara (2015) who observed in the study of blood measurements for different ages of common carp where they increase with the increasing age. Anthony *et al*. (2010) asserts that dummy measurements increase with the age and length of the common carp at the fish ponds of Rasht region at Iran. It has reached 8.2±0.13 mg/dl, 1.75±0.8 cell×10^6mm^3 and 2.31±4.4 cell×10^3mm^3 for Hb, RBC and WBC respectively at three years of age.

Hrubec *et al.* (2001) noted that glucose levels increase with the age of sea bass (*Dicentrarchus labrax*). These measurements are significantly affected by increased length and weight. Farhaad *et al*. (2015) asserts that blood measurements increase with the increase of age in *Ctenopharyngodon idella* (grass carp) and it reached 2.54±0.28 cell×10^6 mm^3, 147±0.61 cell× 10^3 mm^3, 9.9±1.6 g/dl , 164.5±01.26 mg/dl for RBC, WBC, Hb and glucose respectively. Table 6 shows that blood measurements were not different between (males and females) the common carp fish (one group). There also observed an increase in the C group with increasing age and the range of males and females (0.70±0.11 cell×10^6mm^3 and 66±0.13 cell×10^6mm^3), (195±4.6 cell10^3mm^3 and 191 (163±1.961 and 167±1.98 mg/dl) for RBC, WBC, PCV, Hb and glucose for males and females respectively. But they vary between groups for the same sex as they differ between males and females for all the three groups. This result is consistent with the results of Baghizadeh and Khara, (2015). There are no differences between the common-fish species of the common carp fish in the troughs of the Rasht region of Iran. According to Fashaad *et al.* (2015), there are no differences between the grass carp (Orun *et al*., 2003) that the blood measurements differ between species when studying on the three species of carp (*Albunoides bipunctatus*, *Chalcalbumus massulensis*, *Cyprininan macrostomus*) at the Lake Dan, Turkey where it increases in males than in females.

CONCLUSION

Most of blood parameters were affected by age and weight of fish as well as environment temperature. Sex of the fish also had an effect on biochemical profile of blood. Blood parameters of fish differed with changes inaquatic environment and water flow level of Euphrates river in different regions

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