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# Influence of total length, sex and seasonal variations on hematological parameters in *Cyprinus carpio* (Linnaeus, 1758) (Pisces Cyprinidae) in Lake Tonga (Algeria)

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#### ABSTRACT

Hematological parameters are used as an essential tool to assess the health status of fish. This study aims to provide a background on hematology in Lake Tonga's *Cyprinus carpio* (Linnaeus, 1758) (Pisces Cyprinidae) and to demonstrate the impact of seasonal variations, sex and size on hematology. The study was conducted throughout 2018 and involved 120 individuals sampled monthly and randomly. The specimens were weighed and measured. Blood samples were collected to determine hematocrit (Ht), hemoglobin (Hb), red (GR) and white (GB) blood cell count (Lym, Mon, Gra) and erythrocyte constants: mean blood cell volume (VGM), mean blood cell content (TGM), mean corpuscular hemoglobin concentration (CCMH). The results revealed that the majority of the parameters studied did not show significant differences in size classes, and the statistical comparison between the two sexes revealed significant differences in the values of GR, GB, Mon, Mon, Gra, Ht, Hb, TGM and CCMH. On the other hand, all the parameters studied varied significantly over the seasons.

**KEY WORDS** *Cyprinus carpio*; hematological parameters; Lake Tonga; sex; size.

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# INTRODUCTION

Aquaculture in Algeria has become an area of increasing interest to the government and private sector producers, especially breeding activities and the introduction of new fish species.

The common carp *Cyprinus carpio* (Linnaeus, 1758) is a freshwater fish that is common worldwide (Welcomme, 1998). This global distribution of carp is linked to the many introductions around the world to promote aquaculture and sport fishing (Hoffmann, 1995; Copp et al., 2005; Balon, 2006). Fish are closely associated with their environment; physical and chemical changes in the environment are

rapid and uncontrollable and can result in measurable physiological changes in fish (Fazio et al., 2013).

Hematological indices are important parameters for assessing the physiological state of fish and for defining the influence of different environmental factors, pollution or stress on their health (Romestand et al., 1983; Adams et al., 1993; Chen et al., 1995; Houston, 1997). Their changes depend on species, age, sex, sexual maturity cycle and health status (Sniezsko, 1960; Summerfeld, 1967; Blaxhall, 1972; Wedemeyer et al., 1983; Golovina & Trombicky, 1989; Zhiteneva et al., 1989; Bielek & Strauss, 1993; Golovina, 1996; Luskova, 1997; Vosyliene, 1999; Hrubec et al., 2001). Other factors that can significantly affect hematological parameters in teleosts include reproductive cycle, diet, temperature, pH and photoperiod (Sandnes et al., 1988; Kavadias et al., 1996; Svoboda et al., 2001; Guijarro et al., 2003; Kavadias et al., 2003; Bayir, 2005), or stress, pollution, parasitism, size, seasonal variations (Clarks et al., 1979; Barham et al., 1980; Ranzani-Paiva et al., 2004; Camargo et al., 2005; Santos et al., 2009; Onyia et al., 2013; De Souza Neves et al., 2014; Brum et al., 2014; Figueiredo et al., 2014; Fallah et al., 2014).

The aim of this study is to obtain basic information on the haematology of *C. carpio* from Lake Tonga, which is of interest in improving diagnostic and prognostic arguments by regularly monitoring samples from a fish or aquaculture population in order to achieve better control of farming conditions.

#### MATERIAL AND METHODS

#### Study area

Lake Tonga is located in the El-Kala National Park in the extreme north-east of Algeria (36°53' N and 08°31' E). It occupies a vast coastal depression of 2600 hectares with a length of 7.5 km and width

of 4 km (Fig. 1); it has been classified as a World Heritage Site and a RAMSAR site of international importance since 1983. This endorheic freshwater lake is currently the result of various works carried out over the past century and has become a marsh pond, communicating with the sea through an artificial channel, the Messida (Gehu et al., 1993). The Lake Tonga catchment area, with a water volume of about 28,000,000 m<sup>3</sup>, which is significantly higher during periods of high water, includes two major rivers that flow all year round (Oued El Hout, 14 km long, and Oued El Eurg, 10 km long) and an outlet, which is Oued Messida (Bentouili, 2007). The study region is subject to a Mediterranean climate characterized by two different seasons: a humid season, marked by heavy rainfall and low temperatures from October to May, and another dry and hot season with high temperatures reaching their maximum in August (Labar, 2004; Mebarki, 2010).

# Sampling

120 specimens of common carp, *C. carpio* were regularly caught and sampled randomly and monthly from January to December 2017 using eel traps. The total length (Lt, cm) of each fish was measured.

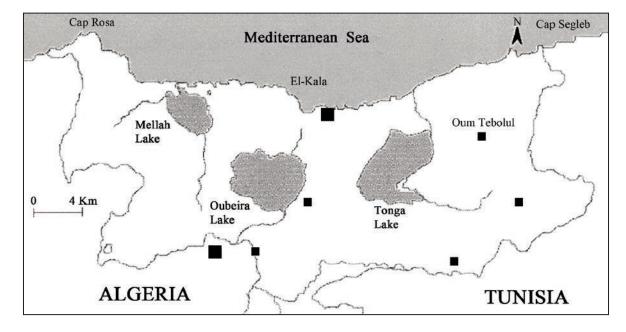


Figure 1. Geographical location of El-Kala National Park (Benyacoub, 1996).

Blood samples were collected immediately on site as soon as the fish were caught by puncture of the caudal vein using a syringe containing a 10% anticoagulant -EDTA.

## Hematological parameters determinations

Hematological parameters were measured by the following traditional analytical techniques: a 540 nm absorbance spectrophotometer using the hemoglobin cyanide procedure for hemoglobin (Hb), by centrifugation of hematocrit capillaries for hematocrit (Ht) and by counting red cells (GR) and white cells (GB) on Thomas cells after dilution in Rees solution (1 g bright cresyl blue, 31.3 g sodium citrate, 10 ml formol 37% and 1000 ml distilled water).

Leukocyte count was performed using stained blood smears with Giemsa / May-Grunwald stain solution (Rosenfeld, 1947); the smears were then examined under an optical microscope (Olympus, Tokyo, Japan) using an immersion oil at a magnification of x100 to obtain the percentage of lymphocytes (Lym), monocytes (Mon) and granulocytes (Gra).

The erythrocyte constants were calculated according to the Wintrobe method (1934) as follows: GMV: mean globular volume (in fl) =Ht (in l/l)/Nb red cells (Tera-/l); GMT: mean globular content (in pg) =Hb (g/l)/Nb red cells (Tera-/l); CCMH: mean corpuscular hemoglobin concentration (in g/) =Hb (g/l)/Ht (l/l).

The data were analyzed by ANOVA for analysis of variance with a significance of 5%, and the means were compared by the Tukey test. The differences were considered significant at  $p \le 0.05$ . Minitab statistical software (version 17) was used for all statistical analyses.

## RESULTS

The total length of the 120 fish studied ranged from 21.4 cm to 61.4 cm (average  $33.73 \pm 8.82$ ). Using Sturges' law, the specimens examined were grouped into 8 size classes of 5 cm in amplitude. The statistical comparison between different size classes revealed that the majority of the parameters studied did not show significant differences (Table 1).

The largest fish (56–61cm) had the highest levels of GR, GB, Hb, Mon and CCMH.

Table 2 shows the results of the hematological indices for males and females. The statistical comparison between the two sexes revealed significant differences in the values of GR, GB, Mon, Mon, Gra, Ht, Hb, TGM and CCMH. Higher amounts of Ht, Hb and Lym were reported in males, while higher values of GR, GB, VGM, TGM, CCMH, Mon and Gra were reported in females. Analysis of the hematological parameters studied for *C. carpio* caught in Lake Tonga waters showed that all values varied significantly over the seasons. In contrast, Hb was the only non-significant parameter (Table 3).

The highest values of Mon, Gra, TGM and CCMH were recorded in the spring. In summer, the number of Lym, GR and Hb reach their maximum. In contrast, the highest numbers of GB and GMVs were observed in winter. Hematocrit is the only parameter that reached its peak in autumn.

# DISCUSSION

Hematology can be used to study fish respon-

	Size classes								
Parameters	21-26 n=31	26-31 n=14	31-36 n=39	36-41 n=10	41-46 n=14	46-51 n=6	51-56 n=3	56-61 n=3	P-value
GR (×106 µl)	1.24±0.24	1.36±0.24	1.27±0.34	1.38±0.37	1.43±0.26	1.38±0.18	1.22±0.08	1.65±0.32	0.177
GB (×103 µl)	64.5±8.75	61.18±9.76	62.54±9.39	61.77±8.83	57.41±7.07	60.78±8.14	61.6±12.44	72.3±5.23	0.184
Ht (%)	19.3±4.53	20.69±5.11	$18.2\pm6.14$	$19.49 \pm 6.92$	23.66±5.09	22.67±5.48	$19.8 \pm 4.4$	19.47±3.73	0.096
Hb (gr/dl)	10.5±1.38	10.95±0.92	$10.91 \pm 1.18$	$11.88 \pm 1.39$	10.07±2.05	$11.06 \pm 0.89$	9.8±1.37	13.16±1.45	0.003**
VGM (fl)	157.89±29.7	151.72±24.69	142.6±23.62	$141.31 \pm 28.71$	166.27±23.26	162.18±20.74	161.5±32.5	118.33±1.29	0.012*
TGM (pg)	85.93±10.89	82.23±14.03	93.36±30.49	91.95±27.07	70.11±6.14	80.45±8.26	79.9±10.41	80.63±7.94	0.045*
CCMH(gr/dl)	55.89±9.32	55.6±12.57	67.94±26.22	68.06±23.43	43.25±8.87	50.97±12.02	52.1±18.9	68.4±6.26	0.001**
Lym (%)	86.2±7.68	87.21±6.45	87.92±5.13	86.73±5.25	88.21±3.16	84.63±4.05	$86.2 \pm 4.84$	84.8±2.99	0.816
Mon (%)	7.98±2.57	7.69±2.7	7.6±2.46	8.25±2.47	7.36±1.95	7.88±1.26	7.37±3.15	10.3±1.24	0.715
Gra (%)	5.78±5.38	5.16±3.93	4.47±2.85	5.02±3.04	$4.42 \pm 1.82$	7.48±2.95	6.43±2.01	4.9±1.74	0.61

Table 1. Comparison of hematological indices in *Cyprinus carpio* from Lake Tonga as a function of total length. P > 0.05 = Not Significant; P < 0.05 = Significant (\*); P < 0.01 = Highly Significant (\*\*).

Parameters	Males n=50	Females n=70	P. Value
GR (×106 µl)	$1.51\pm0.23$	$1.81 \pm 0.26$	<0.001**
GB (×103 μl)	$57.98 \pm 6.93$	$65.48 \pm 9.14$	<0.001**
Ht (%)	$22.54 \pm 5.24$	$17.87 \pm 5.06$	<0.001**
Hb (gr/dl)	$11.13 \pm 1.75$	$10.61 \pm 1.14$	0.054
VGM (fl)	$150.56\pm29.55$	$151.51\pm25.31$	0.85
TGM (pg)	$74.21\pm9.01$	$94.44\pm24.04$	<0.001**
CCMH (gr/dl)	$51.84 \pm 14.15$	$64.6\pm21.66$	<0.001**
Lym (%)	$90.29 \pm 2.93$	$84.27 \pm 6.18$	<0.001**
Mon (%)	$6.45 \pm 1.59$	$8.78 \pm 2.42$	<0.001**
Gra (%)	3.26 ± 1.79	$6.48 \pm 4.13$	<0.001**

Table 2. Comparison of hematological indices between males and females of *Cyprinus carpio* from Lake Tonga. P > 0.05 = Not Significant; P < 0.05 = Significant (\*); P < 0.01 = Highly Significant (\*\*).

siveness to different environmental conditions, which allows a better understanding of their physiology and health status and to develop the most optimal environments for aquaculture. Hematology can also be used in the diagnosis and treatment of diseases (Ranzani-Paiva et al., 2013).

In recent years, the study of fish haematological parameters has received increasing attention and has become a key issue for aquaculture. However, several factors are likely to modify the blood parameters of a fish (size, sex and seasonal fluctuations, etc.).

The findings presented in this study on the effect of seasonal variations, sex and size on hematological parameters in *C. carpio* from Lake Tonga showed that many hematological indices differed significantly with respect to sex and seasonal variations, but were not significant with respect to total length.

The results obtained after the total length effect study show that the rates of GR, GB, Hb, Mon and CCMH were higher in the longest fish. This is consistent with the results of Jawad et al. (2004). The authors found that GR and Hb values increased with increasing fish size. Poston (1966) and Anthony et al. (2010) also observed that the number of GR and Hb tends to increase with the length and age of the fish. As a result, rapid increases in body weight, as well as an increase in blood volume, are accompanied by adequate erythropoiesis (Svetina et al., 2002).

In addition, it may also be the result of changes in plasma or erythrocyte volume (Sandstrom, 1989). However, Chaudhuri et al. (1986) suggested that this relationship may be due to the higher metabolic rate in large fish compared to smaller. Furthermore, Ranzani-Paiva (1995), Svetina et al. (2002) and Baghizadeh & Khara (2015) suggested that the CCMH increased with the age of the *C. carpio* carp, which would be due to adaptation strategies adopted at different life stages.

However, no significant change in hematocrit level relative to total length was observed, despite the general trend observed in the relationship between blood hematocrit and body length of *C. carpio*: the longer the fish, the higher the hematocrit, as reported by Murachi (1959), Svetina et al. (2002) and Hrubec et al. (2001) for mature hybrid tilapias, *Oreochromis* spp. and Orun & Erdeml (2002), in the case of the long spine scraper, *Capoeta trutta* (Heckel, 1843).

GBs are defense cells of the body. According to Douglass and Jane (2010), their levels influence immune responses and the animal's ability to fight infection. White blood cell counts are frequently used as an indicator of health status for fish, as for other vertebrates. These cells are key components of the innate immune defense and participate in the regulation of immunological function in the body (Ballarin et al., 2004). The GB values do not show any significant difference between the size classes. On the other hand, differences in hematological parameters by sex of fish have already been proven (Gabriel et al., 2004; Akinrotimi et al., 2007).

Many studies have shown that males show the highest values in almost all hematological indices; these high values are attributed to greater physiological activity in males (Cech & Wohlschlag, 1981; Orun et al., 2003). Moreover, higher metabolic activity in males may lead to differences in blood cell components (Collazos et al., 1998). Parma & Croux (1994) demonstrated that the variation in hematological indices between the two sexes could be due to differential oxygen demand.

The findings of Fourie & Hattingh (1976) and Baghizadeh & Khara (2015) for *C. carpio* and those of Jawad et al. (2004) for *Tenualosa ilisha* F. Hamilton, 1822 are in agreement with the results of this study, which showed that Hb, Ht and Lym were higher in males than in females.

Whereas, the results of Orun et al. (2003) as well as Orun & Erdeml (2002) for cyprinids were quite the opposite of the results of this study, showing that the number of red and white blood cells in males was lower than in females. Differences in leukocyte count could be attributed in particular to

Parameters	Winter n=30	Spring n=30	Summer n=30	Autumn n=30	P. Value
RG (×106 µl)	1.21±0.18	1.09±0.33	1.53±0.20	1.43±0.23	<0.001**
GB (×103 µl)	67.54±7.31	66.86±9.7	54.07±4.97	60.94±6.51	<0.001**
Ht (%)	19.8±3.64	15.46±4.96	21.95±6.12	22.05±4.96	<0.001**
Hb (gr/dl)	10.71±1.08	11.08±1.25	$10.63 \pm 1.76$	10.89±1.61	0.641
VGM (fl)	163.53±18.8	142.72±23.19	143.13±31.27	$155.08 \pm 28.5$	0.005**
TGM (pg)	89.27±9.83	108.72±27.86	69.31±6.53	76.74±9.33	<0.001**
ССМН	55.4±9.25	8.54±24.33	51.58±15.38	51.61±13.49	<0.001**
(gr/dl)	84.86±5.7	83.05±5.72	91.98±2.26	88.28±4.23	<0.001**
Lym (%)	8.42±2.06	9.78±2.09	5.81±1.36	$7.22 \pm 2.09$	<0.001**
Mon (%)	6.71±4.16	7.15±3.91	$2.20 \pm 1.06$	4.48±2.58	<0.001**
Gran (%)					

Table 3. Seasonal comparison of hematological indices of Cyprinus carpio from Lake Tonga.

(P > 0.05 = Not Significant; P < 0.05 = Significant (\*); P < 0.01 = Highly Significant (\*\*).)

stress, age, maturity, gender, pathogens (biotic factors) and/or water temperature, pH, dissolved oxygen content (abiotic factors) (Pavlidis et al., 2007). As a result, all these environmental variations make interpretation difficult.

The majority of hematological parameters were higher in months of high temperature to meet the considerable energy demand of the fish. They had significantly lower values in months of low temperature due to the body's high metabolic rate due to high body temperature and reproductive activities. These results were not in agreement with the work of Joshi (1989), Orun et al. (2003), Adebayo et al. (2007), Khadjeh et al. (2010) and Kohanestani et al. (2013). As a result, it is recommended that further hematological studies be conducted on the same fish species to obtain further results.

## CONCLUSIONS

This study aimed to provide information on the hematology of *C. carpio* from Lake Tonga (El Kala-Algeria) based on seasonal changes, sex and size. The study was conducted throughout 2018 and involved 120 individuals sampled monthly and randomly.

Seasonal fluctuations were found to have a significant effect on all parameters studied, with the exception of Hb. Sex had a significant effect on the values of GR, GB, Mon, Gra, Ht, Hb, TGM and CCMH. On the other hand, significant differences in the comparison of total length were observed only in the values Hb, GMV, GMT and CCMH. As a result, these results will serve as preliminary data for further studies.

Therefore, it is recommended that further and more detailed research be carried out to determine the effects of the three factors mentioned on hematological parameters in fish, due to the increasing importance given to fish farming and the increased awareness of pollution of aquatic ecosystems.

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