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Hematological Parameters of Brackish Water Fish *Tilapia zillii* in Benghazi, Libya

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عوامل الدم لأسماك المياه المويلحة، البلطي الزيلي في بنغازي، ليبيا.

نادية علي مفتاح الشعافي

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Abstract

Hematology can be a useful tool for monitoring health status, detecting illness, and following the progress of disease and response to therapy. Despite advances in fish medicine in recent years, interpretation of fish hematology often is hampered by a lack of meaningful reference values and the bewildering diversity of fish species. Hematology is the science of studying the anatomical, physiological, and pathological aspects of blood. This study gives valuable information on fish *Tilapia zillii*, Hematological indices (RBC, WBC, Hct, Hb, MCV, MCH, MCHC, GRA, MON, LYM, and PLT) were measured in blood in samples from 30 adult fish captured from (Ain Elmajdob lake), Benghazi city. Statistical analysis revealed that white blood cells were found depending on the length and weight of *Tilapia zillii* as studied by regression of white blood cells on length and weight of *Tilapia zillii*. Moreover, Lymphocytes and Monocytes increased with the increase in weight, as studied by regression of Lymphocytes and Monocytes on the length and weight of *Tilapia zillii*. Nevertheless, not significant in Red blood cells, Hemoglobin, Haematocrit, Mean corpuscular volume, Mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelets.

Keywords: *Ain Elmajdob, Benghazi city, Hematology, Tilapia zillii.*

الملخص

يعتبر علم الدم أداة مفيدة لمراقبة الحالة الصحية واكتشاف المرض ومتابعة تقدم المرض والاستجابة للعلاج. وعلى الرغم من التقدم في طب الأسماك في السنوات الأخيرة، فإن تفسير أمراض الدم في الأسماك غالبًا ما يعوقه الافتقار إلى القيم المرجعية ذات المغزى والتنوع المذهل لأنواع الأسماك. علم الدم هو علم دراسة الجوانب التشريحية والفسيولوجية والمرضية للدم. تقدم هذه الدراسة معلومات قيمة عن أسماك البلطي الزيلي والمؤشرات الدموية (RBC، WBC، Hct، Hb، MCV، MCH، MCHC، GRA، MON، LYM و PLT) في عينة دم مأخوذة من 30 سمكة بالغة تم اصطيادها من (بحيرة عين المجدوب) بمدينة بنغازي. أظهر التحليل الإحصائي أن خلايا الدم البيضاء تعتمد على طول+ووزن البلطي الزيلي كما تم دراسة الانحدار لخلايا الدم البيضاء على طول ووزن البلطي الزيلي. علاوة على ذلك، زادت الخلايا الليمفاوية والخلايا الأحادية مع زيادة الوزن،كما تم دراسة انحدار الخلايا الليمفاوية والخلايا الأحادية على طول ووزن البلطي الزيلي. ومع ذلك، لم يكن معنويًا في خلايا الدم الحمراء، الهيموجلوبين، الهيماتوكريت، متوسط حجم الجسم، متوسط الهيموجلوبين العضلي، متوسط الهيموجلوبين العضلي. تركيز الهيموجلوبين والصفائح الدموية.

الكلمات الدالة: عين المجدوب، مدينة بنغازي، عوامل الدم، البلطي الزيلي.

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1. Introduction

In the broad sense, all the tilapias have in common a mainly herbivorous diet, in distinction to the majority of fishes that feed predominantly on small invertebrates or young or small-sized fishes. They are therefore only one step from the primary producers (plant life) and as they grow to a good size they are a valuable food source for man, the omnivore. The blood transports a variety of materials, including inorganic ions and some organic constituents such as hormones, vitamins, and several plasma proteins.

Cellular constituents of the blood are the red blood cells or erythrocytes, and the white blood cells or leucocytes obtain their characteristic color from hemoglobin. Made up of the colorless protein globin, and the red-yellow pigment heme, which contains iron (Bond, 1979). Recently fish hematological examination is gaining popularity. Several workers have performed hematological studies on fishes; however, data regarding normal health parameters are limited. Few hematologic values of clinically normal tilapia have been reported, but these data may be key for identifying and managing disease issues in recirculating systems (Michael, 2007).

Hematological studies on fishes have assumed greater significance due to the increasing emphasis on pisciculture and greater awareness of the pollution of natural freshwater resources in the tropics. Such studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Chekrabarthy and Benerjee, 1988; and Iwama *et al*., 1976).

Blood tissue reflects physical and chemical changes occurring in organisms, therefore detailed information can be obtained on the general metabolism and physiological status of fish in different groups of age and habitat (Orun and Erdemli, 2003). In fishery, it is important to find out illness and parasites as the source of these causes may not be generally detectable in the early period of the infection, however, it is also possible early diagnosis of illnesses in case of evaluating hematological data, particularly blood parameters (Rimsh and Adamova, 1973).

Although initiated as an adaptive response to destabilizing factors, the physiological stress response can have damaging effects if prolonged. It is well established that continuous stress affects behavior and normal development, with growth reduction (Jobling and Reinsnes, 1986), suppression of reproduction (Gerking, 1980), and increased susceptibility to infections immune-depression, which may cause mortality. Therefore, there have been greater understandings of the need to establish reference hematological and biochemical values in fish to assess health status and the subsequent diagnosis of disease. (Schreck and Bradford, 1990). So, the aims of the present study are; **1)** To obtain a basic knowledge of the hematology of *Tilapia zillii*; and **2)** To evaluate the effect of sex, weight, and length on a selection of hematological parameters in *T. zillii* living in brackish water.

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2. Materials and Methods

2.1. Fish Samples

Thirty Fish of the *T. zillii* were collected by hand net (30 cm in diameter) in December 2009, from (Ain Elmajdob lake), located north Benghazi city. Fish were collected and transported immediately in pouches filled with lake water to the laboratory of the Zoology Department, University of Benghazi. Fish were divided into two groups each group containing (15) fishes, and kept in a laboratory glass aquarium of 200 L capacity were well aerated and provided with external filtration. They were given a minimum period of 72 hrs. to acclimatize to laboratory conditions.

2.2. Water Quality

The water quality parameters such as pH, temperature, dissolved oxygen, and Salinity were measured for two the water systems using a portable meter, (Values are shown in the Results section). The following parameters were measured in the site of collection:
pH: the pH of the water was measured employing the Hanna pH meter.
Water temperature: the water temperature of two ecosystems was measured using the thermometer attached to the water sampler. The temperature reading was recorded after collecting the water inside the sampler with both lids closed. Five minutes time was allowed to elapse before taking the readings after the water samples were lifted out of the water column, to ensure uniformity in the temperature of the water inside the sampler.
Dissolved oxygen of water: was measured using Guard Portable oxygen meter (handy MKI and MKII).
Salinity: salinity of the water was measured employing Hanna meter.

2.3. Blood Collection and Analysis

Random fish samples of *T. zillii* were considered normal on the basis of their external appearance and absence of symptoms of diseases.

The fish sample was caught from the containers. After the preliminary investigation of the length, weight, and sex, a damp cloth was used to cover the fish head. They placed the belly upwards and blood samples were obtained from the heart with the aid of a heparinized disposable plastic syringe containing EDTA as an anticoagulant.

Heart puncture was chosen for being easy to perform and excellent for obtaining a large quantity of blood when applied to Tilapia. After obtaining the desired quantity of blood, the use of a plastic syringe is a necessary precaution with fish blood because contact with glass results in decreased coagulation time (Smith *et al*., 1952). Fish was wiped dry with tissue paper to aid contamination with mucus it was then pushed gently down until blood started to enter in the needle. Thereafter the needle was withdrawn and the blood was gently transferred into heparinized plastic containers, which contained EDTA as an anticoagulant. The samples were then mixed gently.

Then blood samples were transported to Saleem laboratory for analysis using (Diatron instrument) after being adjusted to animal analysis and measurement of Hematocrit (Hct) or

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(PCV), hemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), Lymphocytes (LYM), Monocytes (MON), Granulocyte (GRA), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and of platelets (PLT).

2.4. Statistical Analysis

Results were analyzed with SPSS for windows by using (T-test, Regression).

3. Result

In the study some parameters of water quality were measured (Table 1); the result shows the water quality parameters.

Table 1. The water quality parameters

Water parameters	Result
Temperature	21.4°C
pH	7.7
Dissolved oxygen	3.16 mg/l
Salinity	6.1%

The summary of the mean values of weight, length and hematological parameters for *T. zillii* are represented in Table (2), together with the standard deviation, and Standard Error.

Table 2. Hematological parameters for *T. zillii* in fresh water

Parameter (Unit)	Mean	Standard Deviation	Standard Error	Sig.
Length (cm)	9.76	1.32	0.24	0.558
Weight (g)	18.32	8.65	1.57	0.437
Hb (g/dl)	6.13	1.87	0.34	0.000
Hct (%)	13.56	6.45	1.17	0.010
RBC(10^{12}/L)	0.91	0.33	0.06	0.063
WBC(10^9/L)	53.82	29.18	5.32	0.209
MCHC (%)	42.64	9.53	1.87	0.000
MCH (pg)	63.60	15.24	2.99	0.000
MCV (μ^3)	143.96	29.51	5.48	0.033
MON (10^9/L)	2.02	2.41	0.48	0.000
LYM (10^9/L)	34.96	21.08	4.21	0.003
GRA (10^9/L)	9.52	7.58	1.51	0.001
PLT (10^9/L)	177.10	147.47	26.92	0.122

In the present study, the white blood cells of *T. zillii* were found depending on the length and weight of *T. zillii* studied by regression of white blood cells on the length and weight of *Tilapia zillii*, as well as Lymphocytes and Monocytes, increased with the increase of weight.

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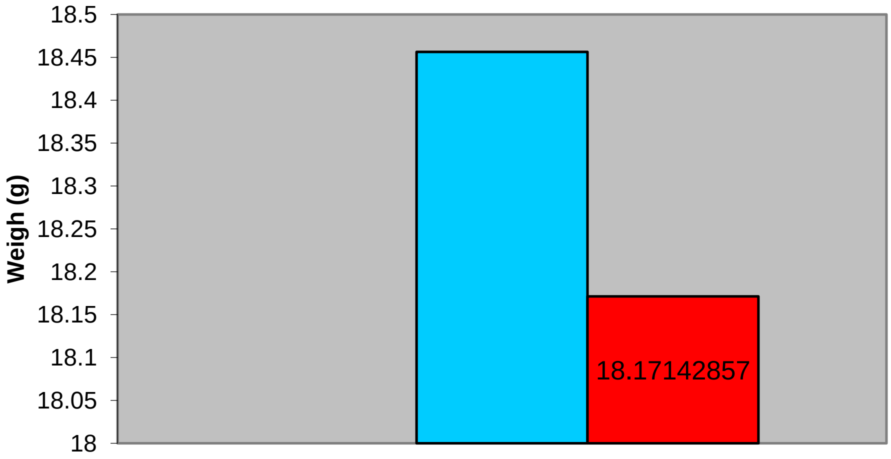

Figure 1. Weight of *T. zillii* fishes from Ain El-Majdob Lake.

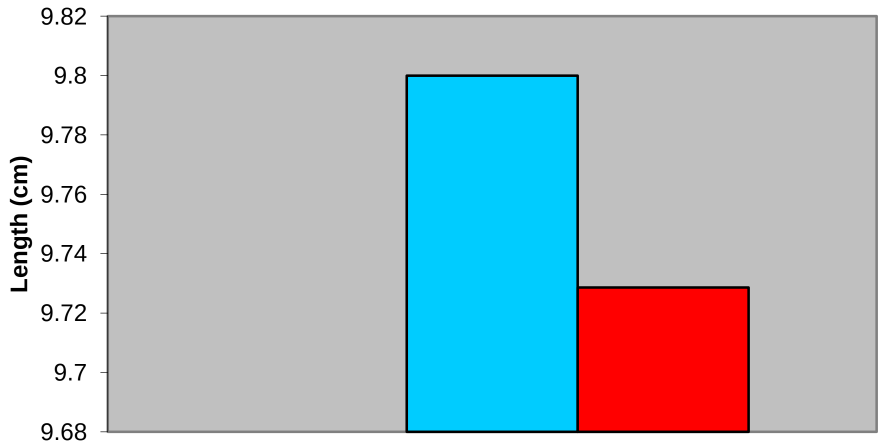

Figure 2. Length of *T. zillii* fishes from Ain El-Majdob Lake.

Table 3. Regression of blood parameters on length and weight of *T.zillii* in fresh water (Red=sig at 0.05)

Blood parameters	Brackish water Length	Brackish water weight
WBC	+	+
LYM	-	+
MON	-	+
GRA	-	+
RBC	-	+
Hct	+	-
Hb	+	-
MCV	+	-
MCH	+	-
MCHC	+	-
PLT	-	+

In this study, the result value of Red blood cells counts of fish was very closely significant RBC is (0.91±0.33) as shown in Figure (3).

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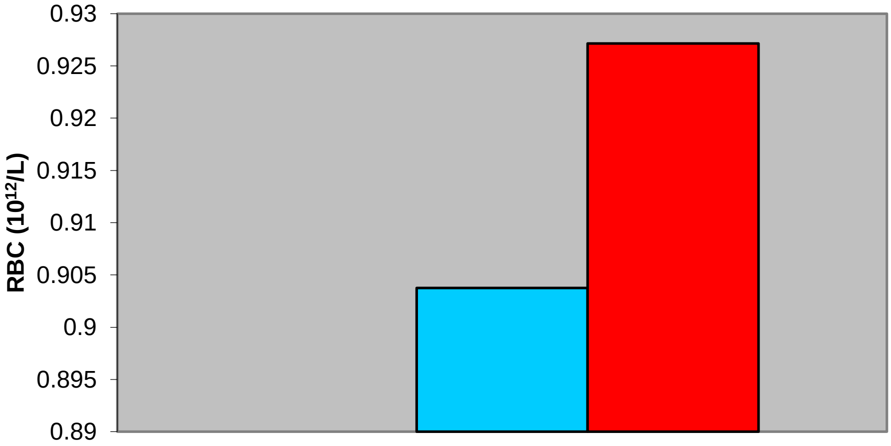

Figure 3. RBC of *T. zillii* fishes from Ain El-Majdob Lake.

The result of hemoglobin concentration for *Tilapia zillii* (6.13±1.87) as shown in Figure (4).

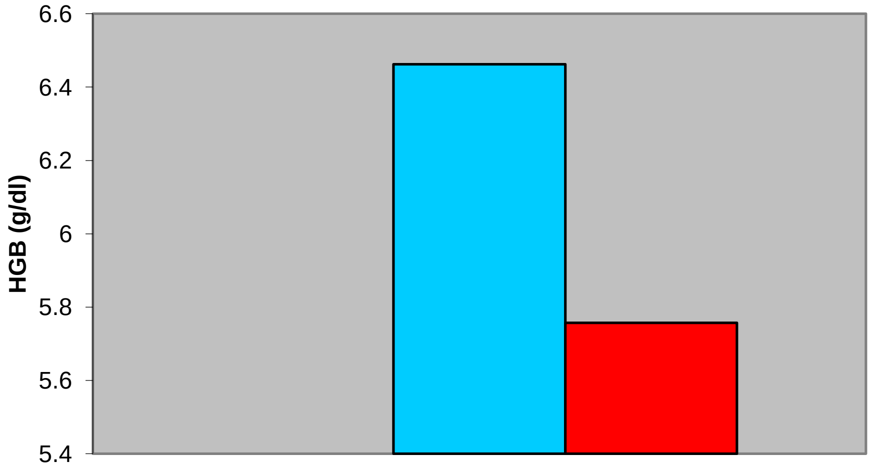

Figure 4. Hb of *T. zillii* fishes from Ain El-Majdob Lake.

The result of hematocrit value (13.56±6.45) as shown Figure (5), and the lymphocyte (34.96±21.08) as shown Figure (6).

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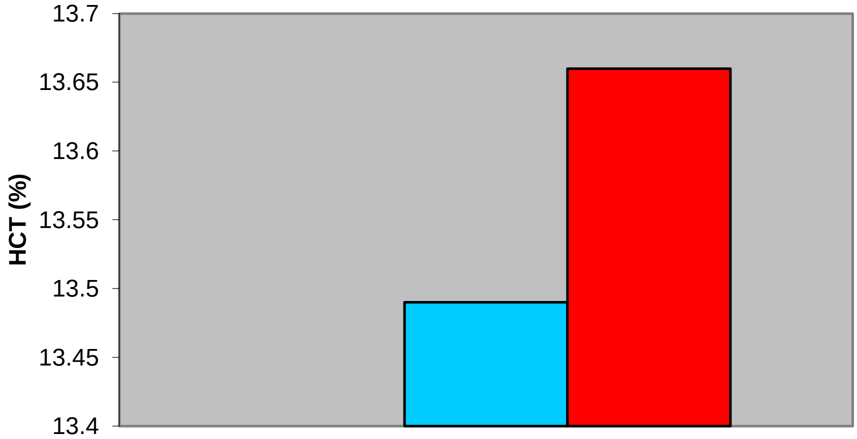

Figure 5. Hct of *T. zillii* fishes from Ain El-Majdob Lake.

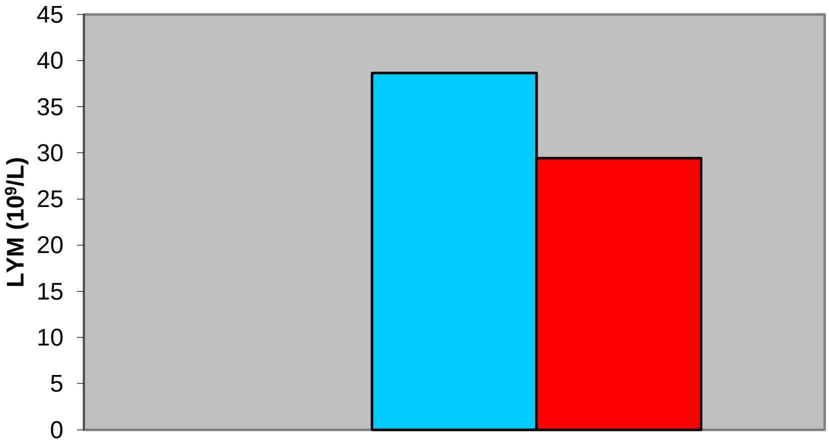

Figure 6. LYM of *T. zillii* fishes from Fish Farm and Ain El-Majdob Lake.

High significant in present result of Monocytes value (2.02±2.44), as shown in Figure (7).

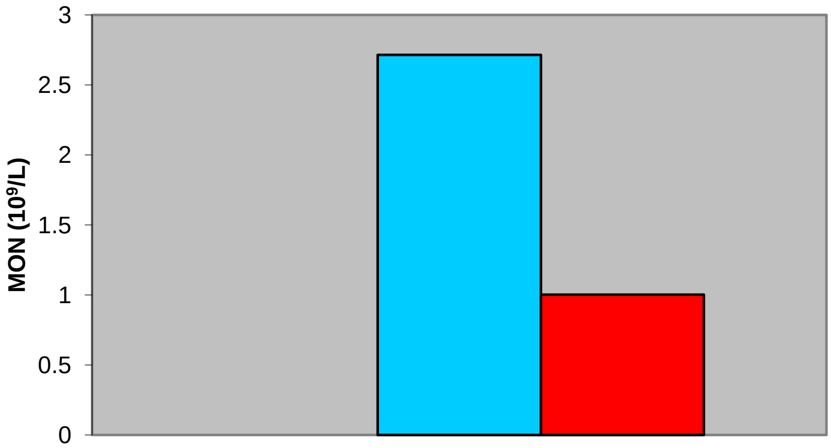

Figure 7. MON of *T. zillii* fishes from Ain El-Majdob Lake.

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In this study, the value of Granulocytes (9.52±7.58), and the value of Mean Corpuscular volume (143.96± 29.51) as shown in Figures (8 and 9) respectively.

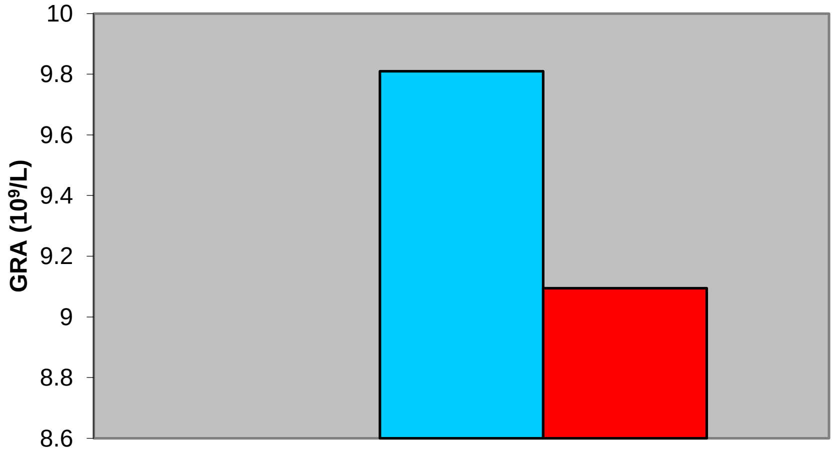

Figure 8. GRA of *T.zillii* fishes from Ain El-Majdob Lake.

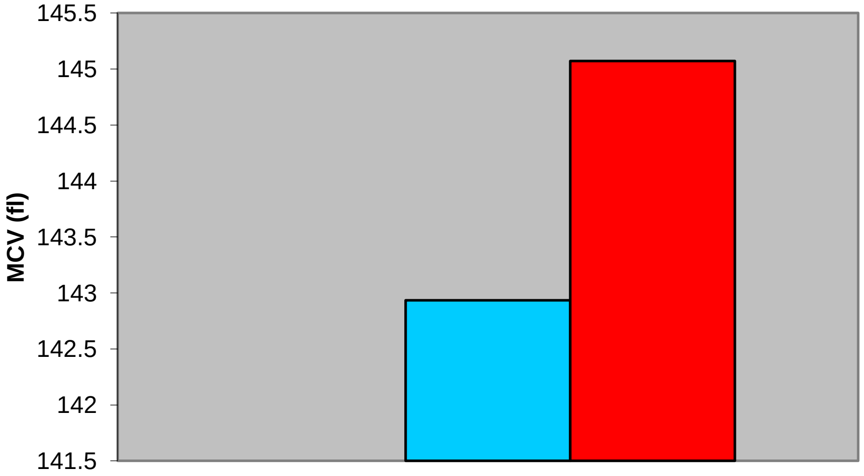

Figure 9. MCV of *T. zillii* fishes from Ain El-Majdob Lake.

The results of this work for Mean corpuscular hemoglobin (63.60±15.24), and Mean corpuscular hemoglobin concentration (42.64±9.53) as shown in Figures (10 and 11) respectively.

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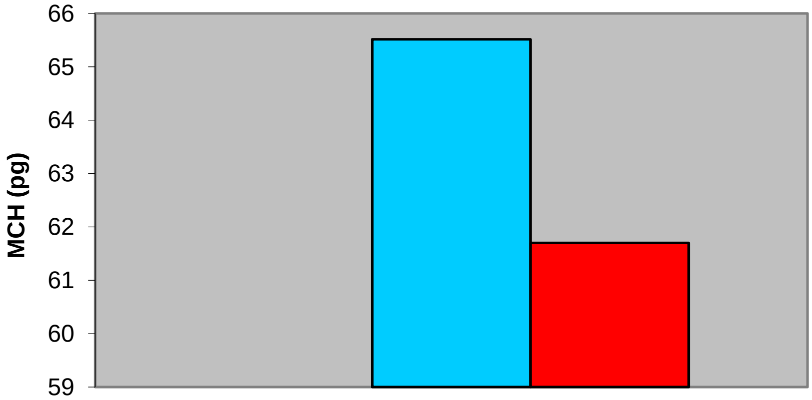

Figure 10. MCH of *T. zillii* fishes from Ain El-Majdob Lake.

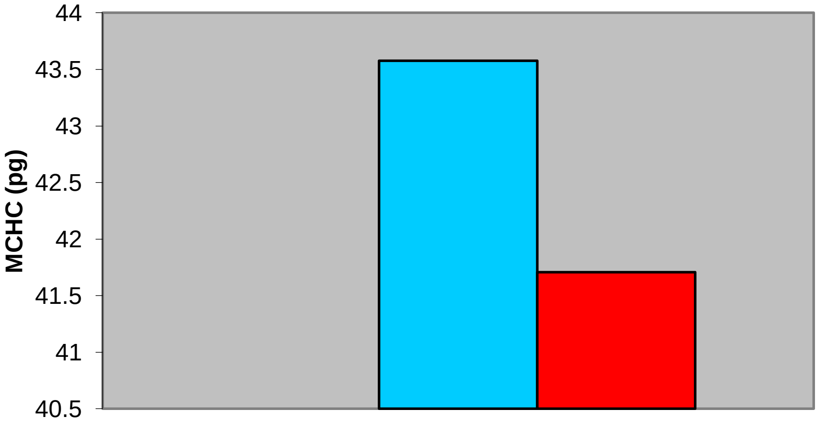

Figure 11. MCHC of *T. zillii* fishes from Ain El-Majdob Lake.

In this work, there was no significant result of the White Blood Cells count, the WBC count of *T. zillii* in freshwater was (53.82±29.18), as shown in Figure (12), and no significant of platelet (177.10 ±147.47) as shown in Figure (13).

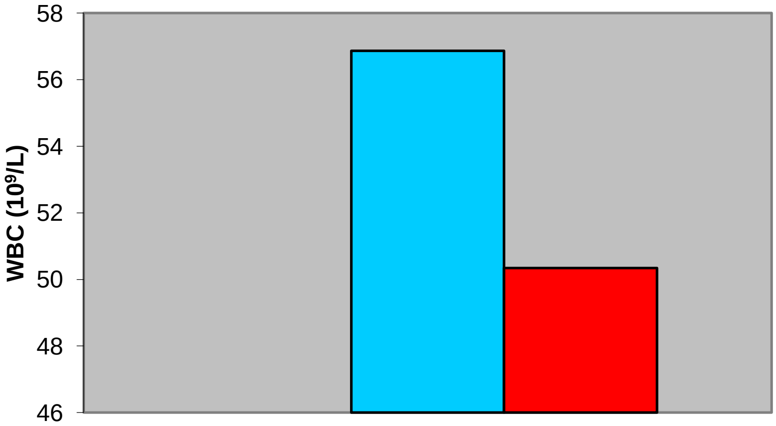

Figure 12. WBC of *T.zillii* fishes from Fish Farm and Ain El-Majdob Lake.

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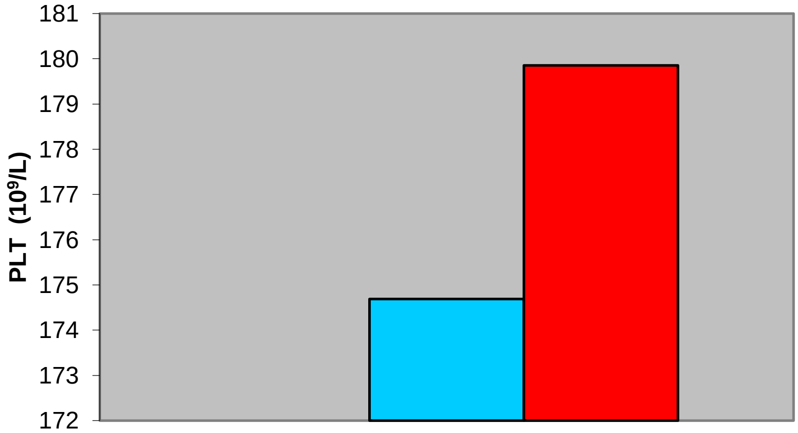

Figure 13. PLT of *T. zillii* fishes from Fish Farm and Ain El-Majdob Lake.

4. Discussion

In this study, the result, such as water temperature was (21.4°C). In shallow water, there may be little or no differences between the temperature at the surface and the bottom. In deep water, temperatures vary with the coolest water at the bottom. Sun *et al.* (1995) concluded that low temperatures have the significant effects of either increasing or decreasing direction on the changes of various blood parameters in tilapias depending on the environmental salinity. An increase in water temperature increased most of the parameters (Ezzat *et al.*, 1973).

In this study, the water pH result was (7.7). The pH value vary depending on a number of factors such as pH levels of the pond water change depending on the aquatic life within the pond, Carbon dioxide produced by aquatic organisms respire has an acidic reaction in the water, the pH in ponds rise during the day as phytoplankton and other aquatic plants remove CO_2 from the water during photosynthesis. The pH decreases at night because of respiration and the production of CO_2 by all organisms. The fluctuation of pH levels will depend on algae levels within the pond. (Aquaculture, 2003).

Also, the Salinity was (6.1%), Salinity is a measure of the amount of salt in water, brackish water is a mixture of fresh and marine water. Salinity significantly caused the decrease of hemoglobin concentrations at 24°C (Sun *et al.*, 1995). High salinity caused an increase in the concentration of most of the blood characteristics, Temperature and salinity changes produce several variations in the blood characteristics of *T. zillii* (Ezzat *et al.*, 1973).
Although the dissolved oxygen was not high (3.16 mg/L) because warm water holds less oxygen than cold water does. Dissolved oxygen in the water is obtained through diffusion from air into water, mechanical aeration by wind or aeration systems, and via photosynthesis by aquatic plants (Aquaculture, 2003).

The weight and length of *T.zillii* in Brackish water between males and females were 16 males weight between (9.4 to 41.9 g) and total length between (7.4 to13.2 cm); 14 females weight between (9.2 to 28.2) g, and total length between (8 to12 cm).

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The effect of length-weight variables on blood parameters such as the enhanced formation of lymphocytes was an essential component of the immune system in the early stages of the growth period (Aldrin *et al*., 1982). In research for Nile tilapia *Oreochromis niloticus*, there was an effect of fish size (between small and big fishes) on all the parameters except for MCV (Tran-Duy *et al*., 2008). In a study on *Mugil* it was verified that mean values for HCT, Hb, MCV, MCH, and MCHC showed a slightly increasing tendency as individuals got larger (Ranzani-Paiva, 1995). Also, larger individuals present higher mean values for MCV, MCH, and MCHC for Dourado *Salminus maxillosus* (Ranzani-Paiva *et al*., 2001).
Al-Hassan *et al*. (1993) observed the hemoglobin and hematocrit values of *Acanthopagrus* latus were having increased with both body weight and length, but to a certain weight and length group after which a slight fall in their value was observed .

In this work the values of RBC of *T. zillii* in brackish water, in this study is similar to *Tilapia zillii* (0.98±0.32) by Ghazaly *et al*. (1992). But lower than control values those reported by Gbore *et al*. (2006) in *T. zillii* from state Government Hatchery (1.10±0.04) when studied the effect of transportation and handling stress on hematology and plasma biochemistry in *T. zillii*, and normal result of *T. zillii* was (3.4±0.23) by Zaki *et al*. (2010a).
The Red Blood Cells have the important function of hemoglobin transport, which carries oxygen to all tissue in the body (Hibiya, 1982). Red Blood Cells count significant decrease may be attributed to the decrease in the erythropoietin activity of the kidney (Santhakumar *et al*., 1999), decreased Red Blood Cells count indicates that the erythrocytes are being affected or destroyed with the infection of fish (Martins, 2008), the reduction in the erythrocyte value may be due to destruction of the Red Blood Cells which might have resulted in the liberation of the hemoglobin content of the cells (Gbore, 2006).
On the other hand, the increased Red Blood Cells count may be due to stimulation of erythropoietin by elevated demands for oxygen or carbon dioxide transport as a result of increased metabolic activity or destruction of gill membranes causing faulty gaseous exchange (Cyria *et al*., 1989). High Red Blood Cells could be explained by the increase in reproductive activity (Collasozn *et al*., 1998). Changes in metabolism and hormonal activity, triggered by cooler water temperatures and declining photoperiod during fall, may result in anemia (Lane, 1979) and reduced erythropoietin production (Zanuy and Carrillo 1985; and Lane 1979).

The hemoglobin within those erythrocytes is the main transport mechanism for oxygen and carbon dioxide in the blood. Alterations in blood oxygen capacity reflect a seasonal adjustment in oxygen transport (Anderson *et al*., 1985; and Cameron, 1970).
The result of hemoglobin concentration in brackish water *T. zillii* is similar to the result of *T. zillii* by Gbore *et al*. (2006), and lower than those described by Zaki *et al.* (2010b), and *T. zillii* by Badawi and Said (1971) when compared the blood of four Tilapia species.
Hemoglobin is the oxygen-carrying component in the blood of fish and its concentration can be used as a good indicator of anemia (Blaxhall and Daisley, 1973). The hemoglobin can vary with season, temperature and nutritional state, and health of the fish (Bond, 1979). The increased hemoglobin content could be explained as a process where the body tries to replace the oxidized denatured hemoglobin (Cyria *et al*., 1989). Changes in the hemoglobin content of

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the blood in response to the environment might come about either by a change in the number of erythrocytes or by a change in the hemoglobin concentration of the individual cells (Anthony, 1961).

Hematocrit provides a measurement of red blood cells (erythrocytes) in whole blood (Anderson *et al*., 1985; and Cameron, 1970). The result of hematocrit value in brackish water *Tilapia zillii* in this study is lower than values obtained from *T. zillii*; 20.07±0.07 (Gbore *et al*., 2006), the result of *T. zillii* 19.80±1.20 (Zaki *et al*., 2010a), and result of *T.zillii* 25.0 (Badawi *et al*., 1971).

The increase of hematocrit value may be attributed to swelling of red blood cells due to increased carbon dioxide in blood, hypoxia, or stress of fish (Ellis,1981), and high hematocrit levels may be caused by metabolic activities (Collasozn *et al*., 1998). These differences may be due to diverse strains used in previous studies and different environmental and cultured conditions. Low temperature caused the decrease of hematocrit (Sun *et al*.,1995).

This work shows a decrease in lymphocytes in *T. zillii* Brackish water contrarily to that observed in *T. zillii* (74.4±2.13) by Ghazaly (1992). However, these values were higher than the values obtained for Nile tilapia (20.5±9.7) by Martins *et al*. (2008), and values of *Tilapia guineensis* (35.46±4.11) by Akinrotimi *et al*. (2010).

The result of Monocytes value in brackish water *T. zillii* in this research was lower than those studied by Martins *et al*. (2008) in Nile tilapia (14.3±7.7) and *Tilapia guineensis* (3.12±1.01) by Akinrotimi *et al.* (2010). High Monocytes could be explained by the increase in reproductive activity and defense mechanism of fish (Orun and Erdemli, 2003).

The result of Granulocytes of *T. zillii* in this research is lower than the value referred by Akinrotimi *et al.* (2010) in *Tilapia guineensis* (20.95±2.21), but higher than the value obtained for Nile tilapia (6.5±4.6) by Martins *et al*. (2008), and *T. zillii* (4.8±1.18) by Ghazaly (1992). Granulocytes are phagocytic, involved in combating disease, and may increase in number when the fish is infected by bacteria (Bond, 1979).

The lower value of Mean Corpuscular volume was found in the present study in *T. zillii* than those referred by Bittencouret *et al.* (2003) in Nile tilapia (148.80±153.19) and the result of *T. zillii* (181.78±0.06) by Gbore *et al.* (2006). Nevertheless, the value of Mean Corpuscular volume in the present work *T. zillii* was higher than the value obtained in Tilapia guineensis; 80.76±2.11 by Akinrotimi *et al*. (2010), and *T. zillii* 104.29±13.7 (Ghazaly, 1992). Changes in the hemoglobin content of the blood in response to the environment might come about either by a change in the number of erythrocytes or by a change in the hemoglobin concentration of the individual cells (Anthony, 1961).

The value of MCH and MCHC in this work were higher than the values; MCH value was (23.96±1.21), and MCHC value (29.66±2.21) that obtained in *Tilapia guineensis* (Akinrotimi *et al*., 2010). MCH (46.48±2.49), MCHC (33.14±1.88) in *T. zillii* by Gbore *et al*. (2006) and values MCH (40.74±34.19), MCHC (35.24±14.92) that concluded in Nile tilapia (Bittencouret *et al*., 2003). The increase of Mean corpuscular hemoglobin concentration may be attributed to swelling of red blood cells due to increased carbon dioxide in blood, hypoxia, or stress (Ellis, 1981).

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In this work, there was no significant result of the White Blood Cells count. The White Blood Cells are known to be involved in the regulation of immunological functions of the body and animal resistance to some vulnerable diseases. Decrease of white blood cells to protective functions of the body (Santhakumar *et al*., 1999). The White Blood Cells of the blood respond to various stressors including infections and chemical irritants (Mohammed and Sambo, 2010). The significant change in the white blood cells values of *Tilapia zillii* might be due to the stress of handling and transportation (Gbore *et al*., 2006).

In this work, the results of platelet were no significant *T. zillii*. These values are higher than the platelet value (40.02±3.14) that was studied by Akinrotimi *et al*. (2010) in *Tilapia guineensis*.

Platelet is involved in blood clotting, they carry a chemical that promotes the conversion of prothrombin and thrombin (Bond, 1979). Nowadays, thrombocytes have been found to be an important cell involved in fish defenses (Martins *et al*., 2008).

Finally, differences in levels of some hematological parameters could be explained with the effects of body size and ecological conditions. The increase in hematological parameters might result from the difference in water temperature and oxygen concentration (Orun and Erdemli, 2003). An attempt was made to compare the reference range for hematological values for *T. zillii* and the range of the corresponding values for *T. zillii* specimens reported by other investigators. A direct comparison was not possible because of the different experimental factors, there is a wide range of physiological variations are influenced by environmental conditions, gender, origin, feeding, and age (Bittencouret *et al*., 2003).

5. Conclusion

In conclusion, Hematological indices (RBC, WBC, Hct, Hb, MCV, MCH, MCHC, GRA, MON, LYM, and PLT) were measured in one blood sample from 30 adult fish captured in brackish water (Ain El-Majdob lake). Compared to most teleosts, *Tilapia zillii* has similar mean values for MCV and Hb and lower for RBC and Hct, and higher for other blood parameters. Statistical analysis revealed that differences in hematological parameters between male and female fish were not significant. This study gives valuable information on fish *T. zillii* Therefore, we propose separate data collection and comparison from healthy and unhealthy fish to obtain reliable hematological data. However, the result of the present study may be helpful in obtaining standard values of blood parameters.

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