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SEASONAL VARIATIONS IN SOME HEMATOLOGICAL TRAITS OF *TILAPIA ZILLII* (GERVAIS, 1848) IN A SOUTHERN MEDITERRANEAN SEA BRACKISH LAGOON

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ABSTRACT: The aim of this study was to determine seasonal variations in CBC, and liver and kidney functions of *Tilapia zillii* of Ras Al-Tin brackish lagoon, eastern Libya, Southern Mediterranean Sea, during 2019/2020. During this period, the seawater temperature averaged 23^0C; the minimum was in winter and the maximum in summer. Salinity was 4.67 to $23^0/_{00}$, the lowest was in winter, and the highest in summer. Conductivity was 11 to 27mS/cm**.** Dissolved oxygen was minimal in summer (9.5mg/L) and maximal in autumn and spring (11.9 and 11.4mg/L consecutively). In summer and autumn, the minimum and maximum WBC and RBC counts were 153.6 and 211.9 (103/L), respectively, and 0.798 and 1.7 (106/L). Hb was highest in winter, while HCT was highest in autumn. The effect of season on MCV, and PLT was insignificant. MCH and MCHC were highest in winter. Strong correlations occurred between Hb/RBC, HCT/RBC, HCT/Hb and MCHC/MCH. Minimum and maximum GOT activities were 90.6 in winter and 160.3U/L in autumn, for GPT, the activities were 12.0 in spring and 25.6U/L in autumn. ALP levels were lowest in the winter (83.8mg/L) and highest in the summer (112.4mg/L). LDH activity was lowest in autumn (291.3 mg/L) and highest in spring (1331.0 mg/L). Urea was high in spring, while uric acid was highest in autumn (4.33mg/dL). The effect of season on creatinine was insignificant. Strong correlations occurred between LDH/Urea and LDH/GPT. All the above hematological variations were attributed to seasonal changes in the habitat conditions. However, these conditions were within the range favorable for the thriving and flourishing of *T. zillii.*

KEYWORDS*:* Seasonal variations, hematological traits, Tilapia zillii, southern Mediterranean Sea, brackish lagoon

INTRODUCTION

Carps, salmonoids, and tilapias are the most important cultured fish worldwide (FAO, 2020). Tilapias, originally a tropical African fish of about 70 species (Meyer, 2002), are now cultured in more than 85 countries (Fitzsimmons, 2000), where total production was 6.5 mt in 2019 and is rising. Tilapia is a robust, medium-sized euryhaline fish that can adapt to a wide range of temperatures, and low oxygen concentrations. It breeds in captivity and accepts a wide range of artificial feed; its food conversion ratio and growth rates are high, and the fish is highly tasty (Asad *et al*., 2010; Mohamed, 2018; Ali, 2019).

Tilapia zillii and *Oreochromis niloticus* were introduced into many of the brackish coastal lagoons scattered on the eastern coast of Libya (southern Mediterranean Sea), where they are now contributing significantly to the artisanal catch (Reynolds, 1995; Mohamed, 2018; Ali, 2019).

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Seasonal changes in habitat parameters are known to affect the hematological traits of fish (Behat, 2017; Forghaly *et al*., 1973; Fredianelli *et al*., 2018). The objective of the present work was to establish the main attributes of the blood (complete blood count and liver and kidney function indicators) of *T. zillii* in Ras Al-Tin (Eastern Libya) as indicative of the Southern Mediterranean Sea brackish lagoons.

METHODS

Description of the study area

All Tilapia fish used in the present study were collected from (32°37'00.0"N 23°07'00.0"E), a relatively small, shallow, sandy, brackish lagoon (Fig. 1 and 2) typical to those found scattered along the southern Mediterranean Sea coast of Libya. Ras Al-Tin is located within the Gulf of Bumba (Fig. 1) where a frontal reef barrier provides shelter for the artisanal fishing boats of the lagoon (Fig. 2) (Reynolds *et al*., 1995; Abd AL Hamid *et al*., 2017).

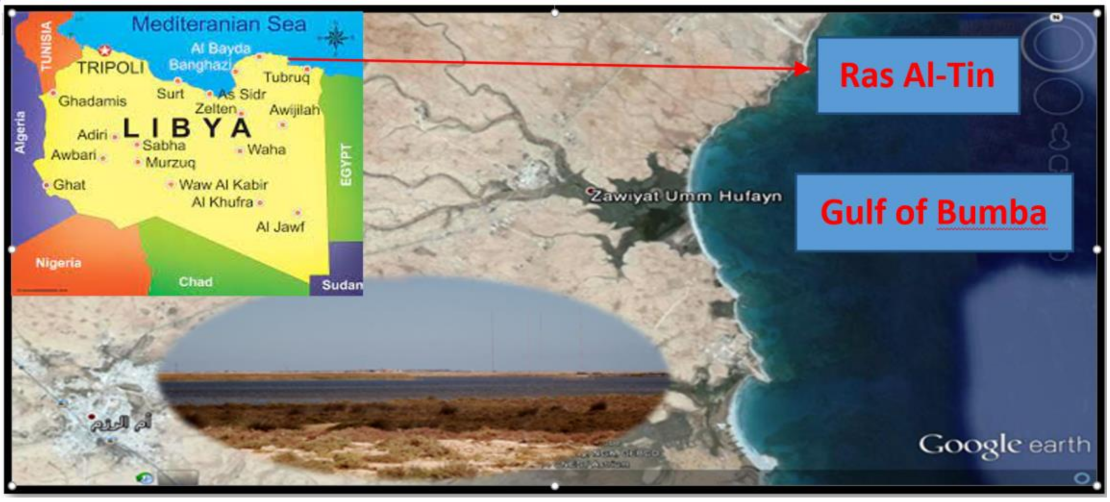

Fig. 1. Ras Al-Tin (Google image).

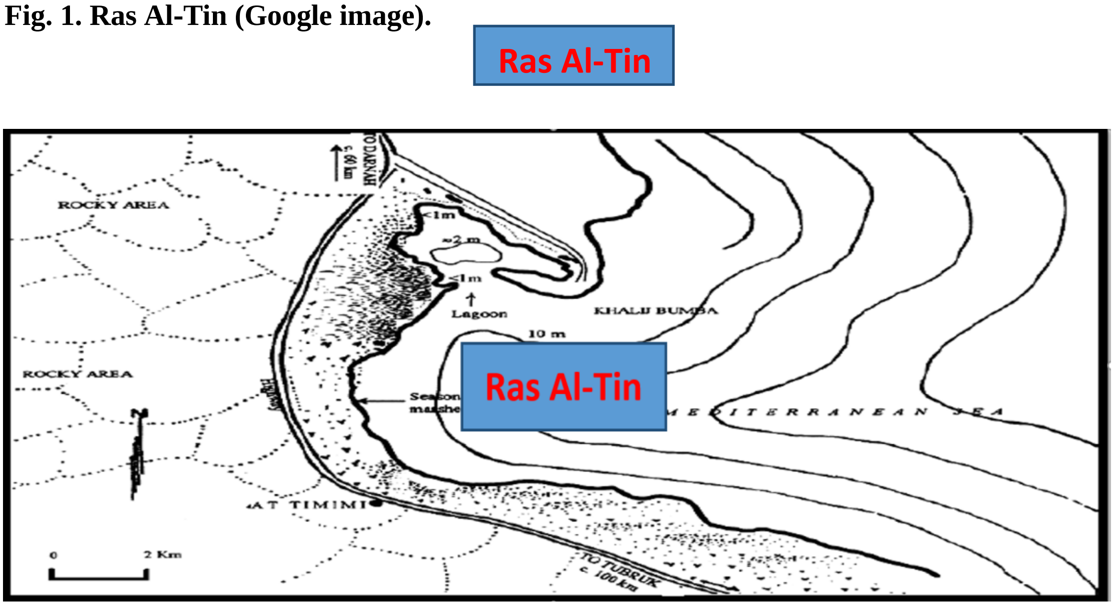

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Fig. 2. Ras Al-Tin lagoon, Eastern Libya.

Measuring the physical-chemical properties of sea water at the study site

Temperature, salinity, conductivity, and dissolved oxygen of seawater were measured with probes in Ras Al-Tin at midday following the collection of fish samples.

The collection of fish samples

Sixty live adults *Tilapia Zillii* were collected from Ras Al-Tin during the winter, autumn, spring, and summer seasons of 2019/2020 and immediately placed in a basin containing seawater treated with clove oil as an anesthetic.

Collecting the blood samples

Blood was rapidly drawn from the caudal vessel or heart of each fish using sterile disposable plastic syringes fitted with 21-gauge needles (Hrubec *et al.*, 1997) and stored in conventional blood vials treated with EDTA as an anti-coagulant, and in non-treated vials for obtaining the serum. For serum preparation, blood was allowed to clot on ice for 1 h. Serum was separated from whole blood by centrifugation at 3000 g for 5 min. The vials were then taken to the medical laboratory for determination of liver and kidney function indicators. The fresh blood (nonclotted blood) was introduced into the XP-300, an automated 3-part differential hematology analyzer.

Measuring the CBC parameters and the liver and kidney functions

In the laboratory, the CBC parameters (WBC, RBC, Hb, HCT, MCV, MCH, MCHC, PLT) were measured with Sysmex XP-300 Hematology Analyzers, and the livre (GOT, GPT, ALP, LDH), and kidney (urea, uric acid, and creatinine) functions were measured spectrophotometrically using 4040 V5 photometer according to Patton and Crouch (1977) and Henry *et al*. (1974).

RESULTS

Characteristics of the surface water of Ras Al-Tin lagoon

During the one-year study period, temperatures ranged from 11 to 23 Celsius (Table 1). The minimum was in winter and the maximum was in summer. Salinity ranged from 4.67 to 23$^{0}/_{00}$, reflecting the brackish nature of the lagoon. The minimum salinity was in winter; the maximum was in summer. Conductivity ranged from 11 to 27mS/cm**.** Dissolved oxygen ranged from a minimum of 9.5 in summer to a maximum of 11.9 and 11.4 in autumn and spring, respectively.

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Table 1. Characteristics of the surface water of Ras Al-Tin lagoon (mean±SE).

Season / Parameter	Summer	Autumn	Winter	Spring
Temperature ^{0}C	**23±0.00**	**18±0.00**	**11±1.73**	**14±1.15**
Salinity $^0/_{00}$	**23±1.15**	**12±.58**	**4.67±.88**	**9±1.732**
Conductivity mS/cm	**27±1.73**	**24±1.73**	**11±1.15**	**17±1.15**
Oxygen mg/l	**9.5±0.00**	**11.9±0.00**	**10.7±0.00**	**11.4±0.00**

Descriptive statistics of the fish used in the present study

The mean values of total length (TL± St E) and total weight (TW± St E) of *Tilapia zillii* used in the present study were 16.80±1.029cm and 227.26±53.307g (Table 2). The smallest fish was 9.00cm (122g), and the largest was 22.00cm (700g).

Table 2. Descriptive statistics of the fish used in the present study

	Minim.	Maxim.	Mean	St E
TL cm	**9.00**	**22.00**	**16.80**	**1.029**
TW g	**122.00**	**700.00**	**227.26**	**53.307**

The complete blood count (CBC) parameters of *T. zillii*

Individual CBC parameters by seasons are presented in Figs. 3 to 10. The mean WBC in summer, winter, and spring (Fig. 3) were not significantly different. The winter and summer means differed significantly from the autumn means. The highest WBC count was in the autumn. RBC counts during summer, winter, and spring were not significantly different from each other (Fig. 4). Autumn, winter, and spring means were not significantly different; autumn and summer counts were different. Summer, autumn, and spring Hb were not significantly different (Fig. 5). The highest value was in winter. Autumn, winter, and spring HCT (Fig. 6) were not different. Summer, winter and spring values were also not different. Autumn and summer values were significantly different. but summer and winter counts were not. The highest value was in autumn. MCV, and PLT means of all seasons (Fig. 7 and 10) were insignificantly different. Summer, autumn and spring MCH, and MCHC (Fig. 8 and 9), means were indifferent, but were different from winter mean. The highest value was in winter.

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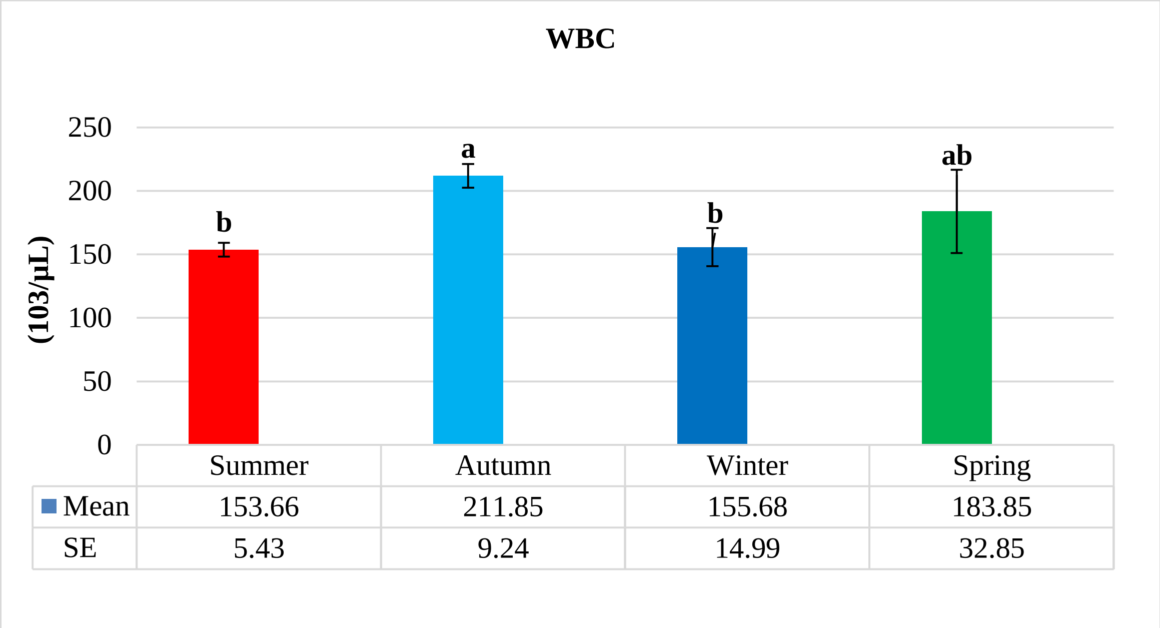

Fig. 3. Mean ± St E WBC count of *T. zillii* during summer, autumn, winter, and spring. Different superscripts indicate different means.

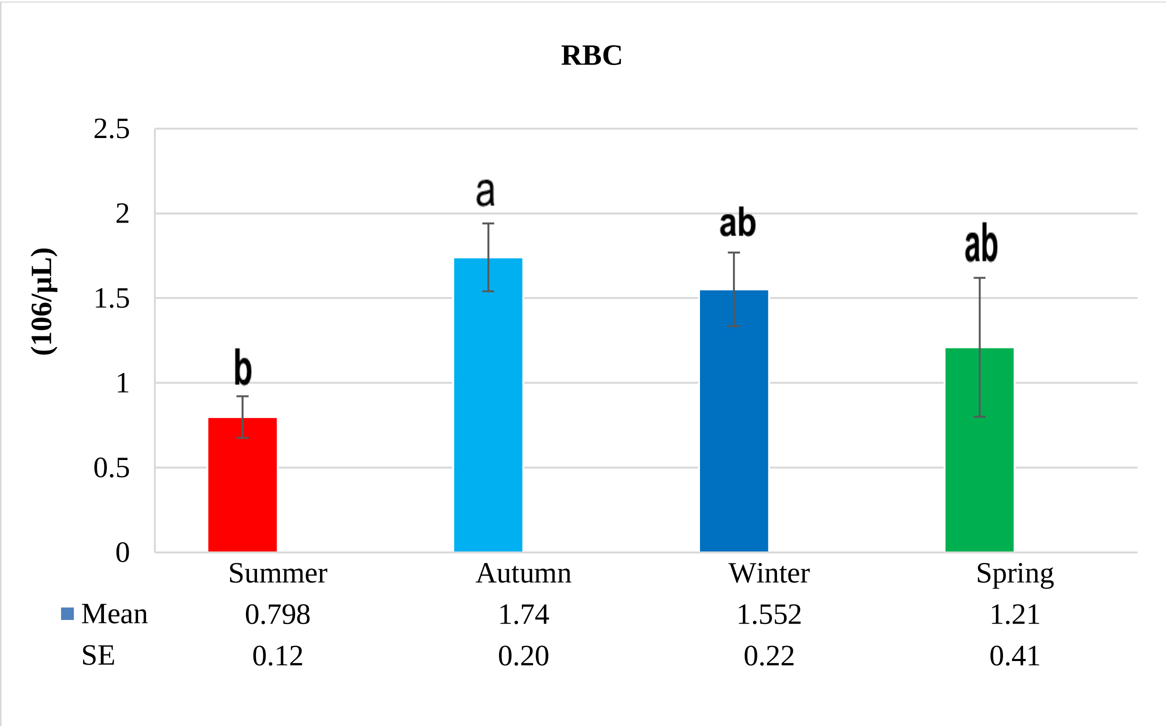

Fig. 4. Mean ± St E RBC count of *T. zillii* during summer, autumn, winter, and spring. Different superscripts indicate different means.

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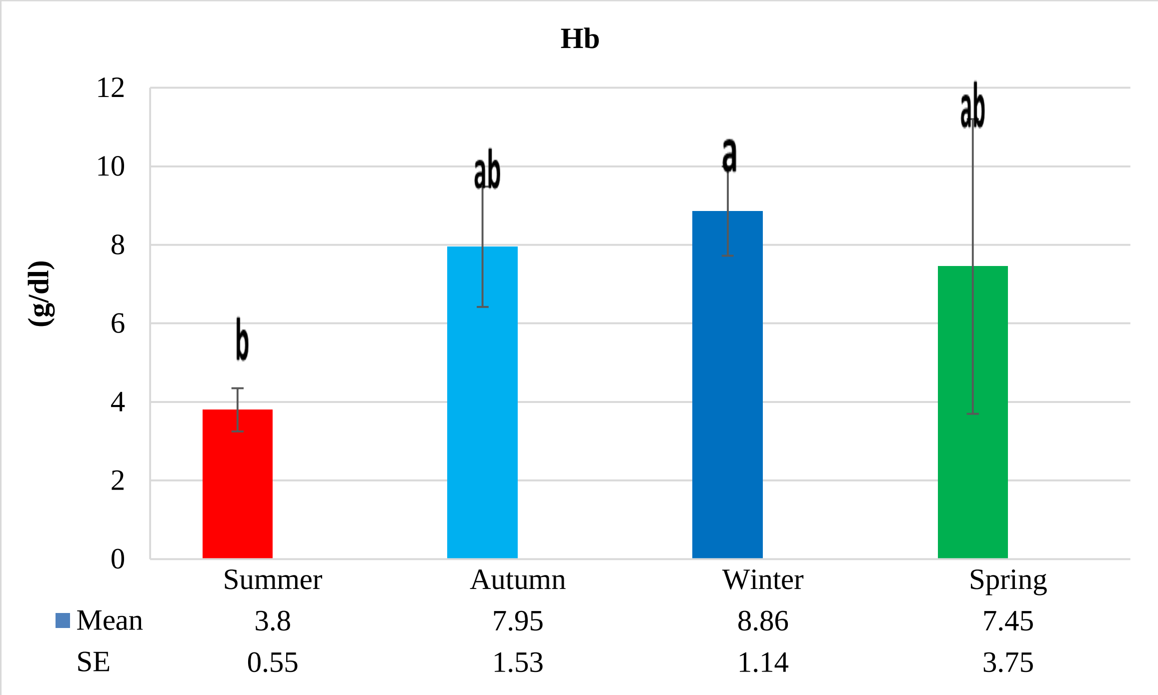

Fig. 5. Mean concentration ± St E of Hb of *T. zillii* during summer, autumn, winter, and spring. Different superscripts indicate different means.

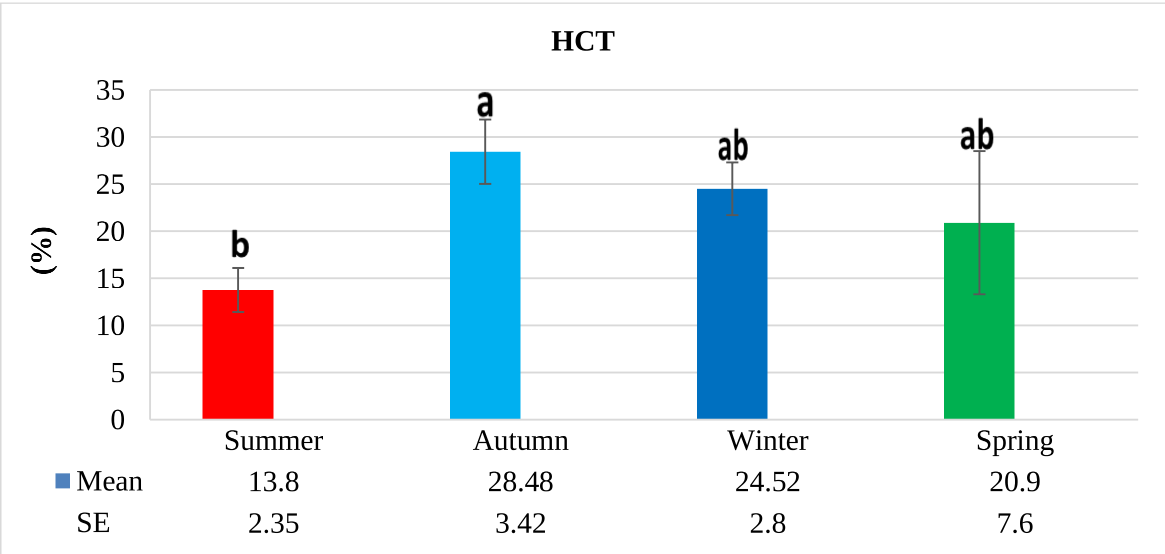

Fig. 6. Mean ± St E of HCT of *T. zillii* during summer, autumn, winter, and spring. Different superscripts indicate different means.

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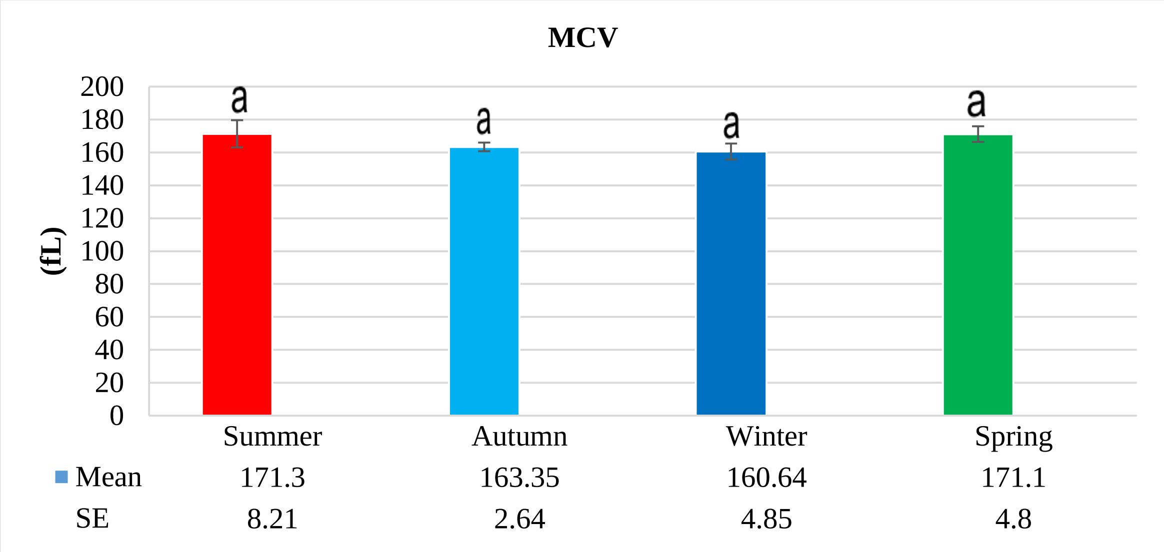

Fig. 7. Mean ± St E of MCV of *T. zillii* during summer, autumn, winter, and spring. Different superscripts indicate different means.

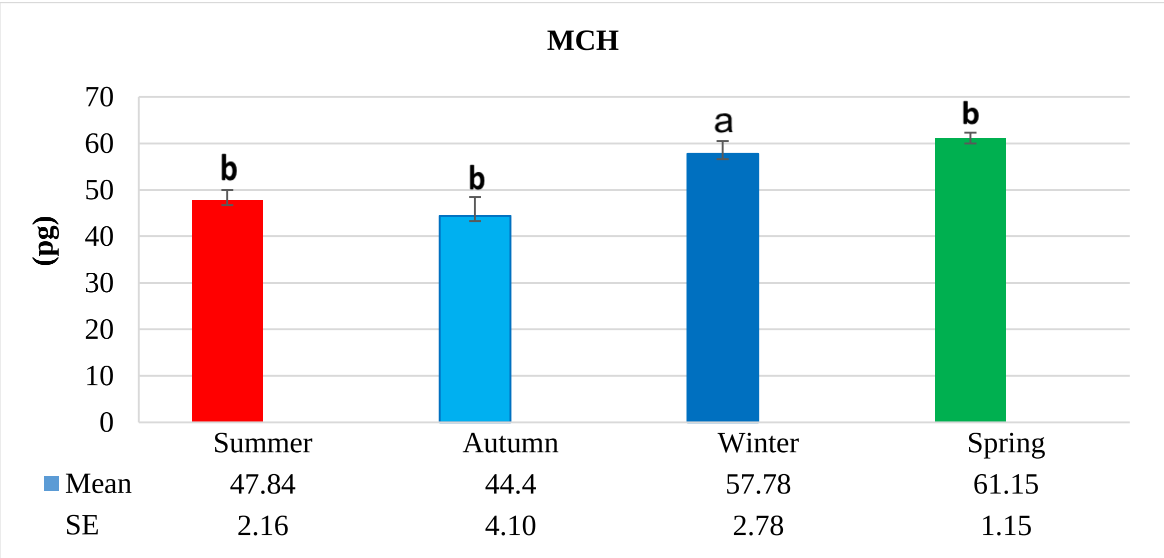

Fig. 8. Mean ± St E of MCH of *T. zillii* during summer, autumn, winter, and spring. Different superscripts indicate different means.

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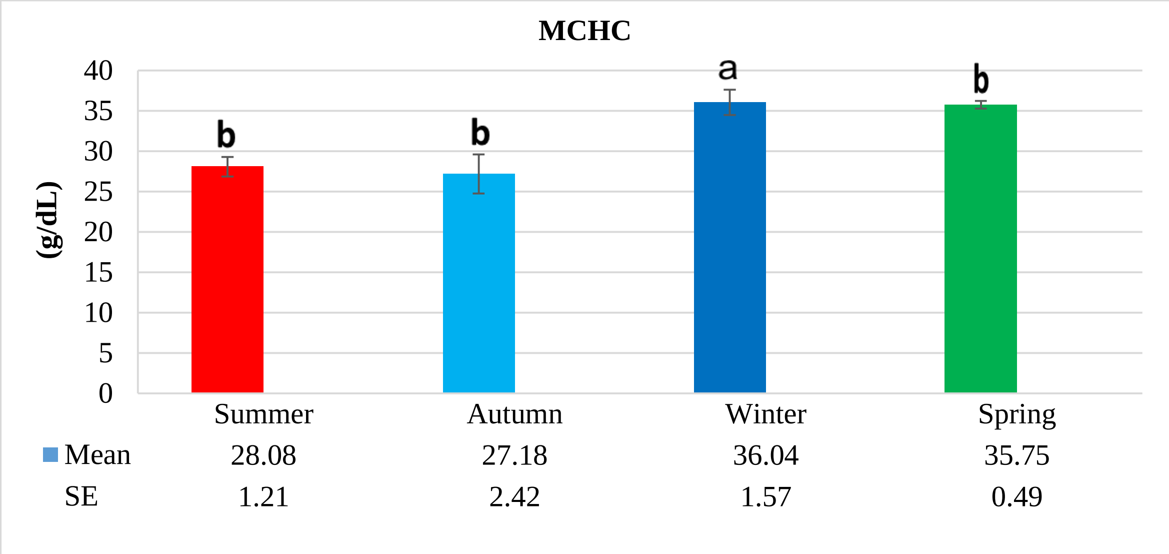

Fig. 9. Mean ± St E of MCHC of *T. zillii* during summer, autumn, winter, and spring. Different superscripts indicate different means.

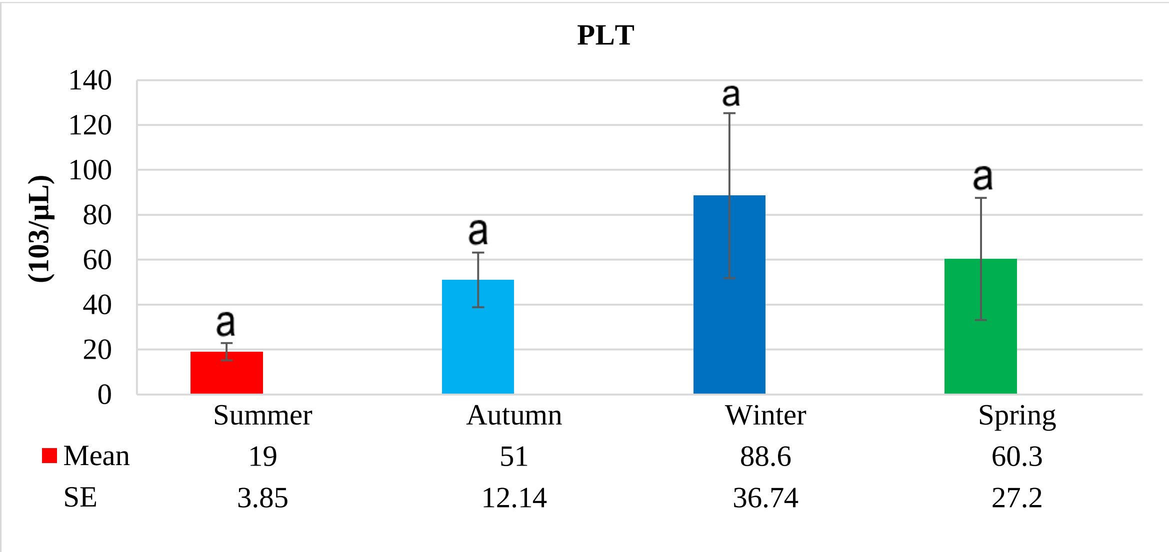

Fig. 10. Mean ± St E of PLT of T. zillii during summer, autumn, winter, and spring. Different superscripts indicate different means.

Binary correlation between CBC parameters of *T. zillii*

Strong (high correlation coefficient: r) and significant ($p \leq 0.05$ or 0.01) correlations were observed between the following CBC pairs (Table 3): RBC/HCT: 0.985**, RBC/Hb: 0.972**, Hb/HCT: 0.964** and MCH/MCHC: 0.834**. Medium to moderate correlations occurred between Hb/MCHC: 0.670*, WBC/HCT: 0.602*, WBC/RBC: 0.587* and WBC/Hb 0.587*. Correlations between the other CBC pairs were insignificant.

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Table (3). Pearson's binary correlation between CBC parameters of *T. zillii*.

	WBC	RBC	Hb	HCT	MCV	MCH	MCHC
RBC	.587*						
Hb	.587*	.972**					
HCT	.602*	.985**	.964**				
MCV	-.102	-.359	-.324	-.212			
MCH	.130	.321	.521	.354	.113		
MCHC	.191	.514	.670*	.461	-.451	.834**	
PLT	-.159	-.136	-.032	-.166	-.070	.459	.453

(n = 15; *: P ≤ 0.05; **: P≤ 0.01)

The kidney and liver functions parameters of *T. zillii*

Mean GOT activity in summer, winter and spring (Fig.11) were not significantly different, autumn activity was not different from summer and spring activities, but differed from winter activity. GPT winter and summer means differed significantly from autumn means (Fig. 12). The highest value was in autumn. Spring and autumn means were significantly different. ALP activities during summer, autumn and spring (Fig. 13) were not significantly different from each other; summer and winter means were significantly different. The highest value was in summer. LDH autumn and winter activities were not significantly different (Fig. 14), but differs from concentrations of summer and spring which were significantly different from each other. The highest activity was in spring. Summer, autumn, and winter. Urea concentrations were not significantly different but were significantly lower than spring activity (Fig. 15). Autumn, winter and spring uric acid activities were not significantly different from each other (Fig. 16), also summer and spring activities were indifferent. Autumn and winter means were different from those of summer. The highest value was in autumn. Creatinine concentrations were statistically similar in all seasons (Fig. 17).

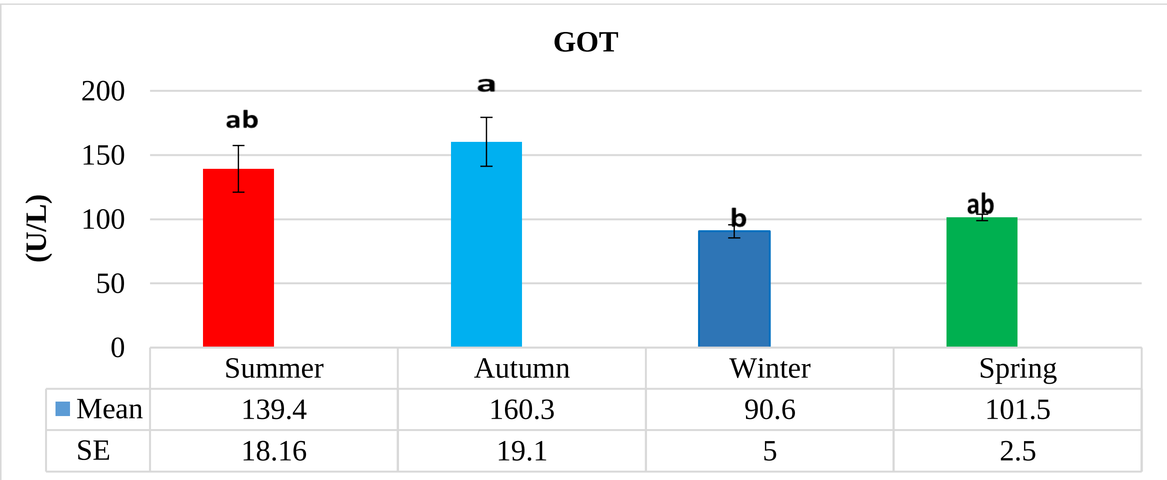

Figure. 11. Mean activity ± SE of GOT of *T. zillii* during summer, autumn, winter and spring.

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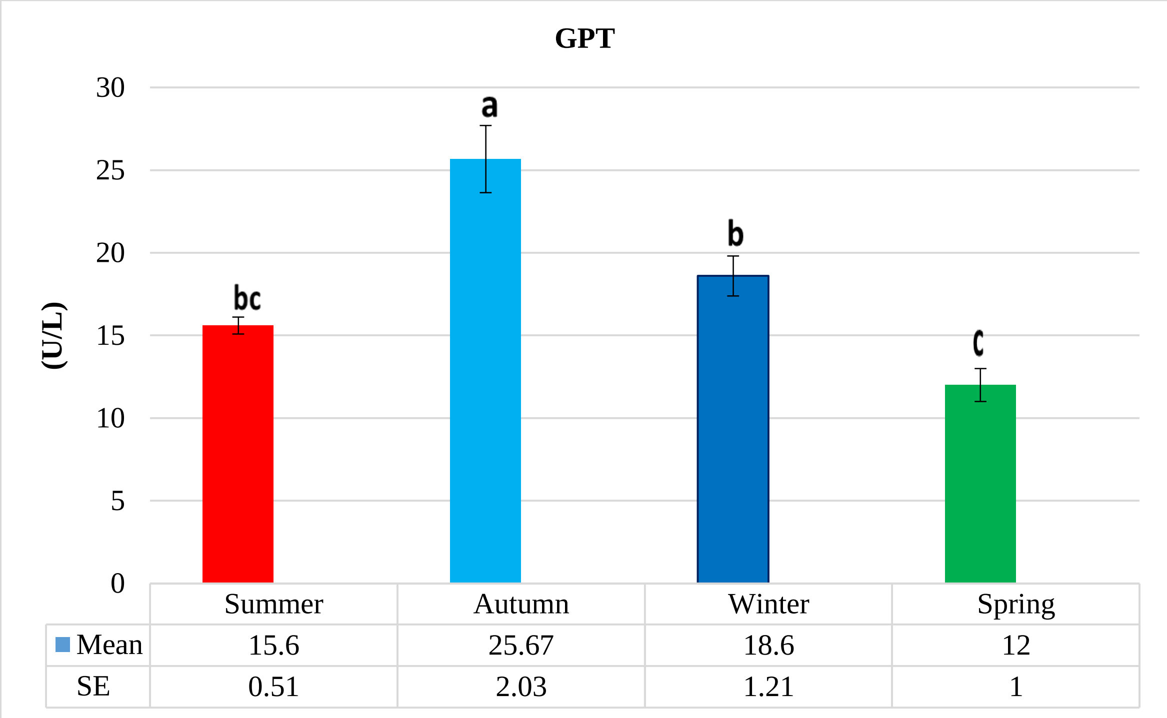

Figure. 12. Activity ± SE of GPT of *T. zillii* during summer, autumn, winter and spring

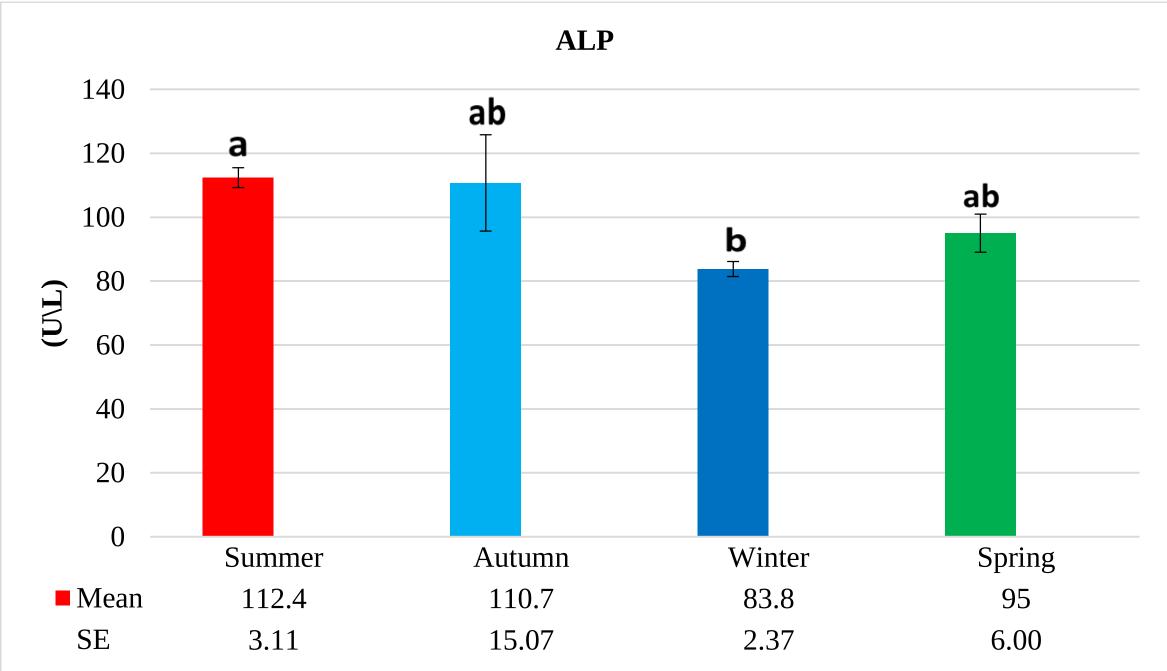

Figure. 13. Mean activity ± SE of ALP of *T. zillii* during summer, autumn, winter and spring.

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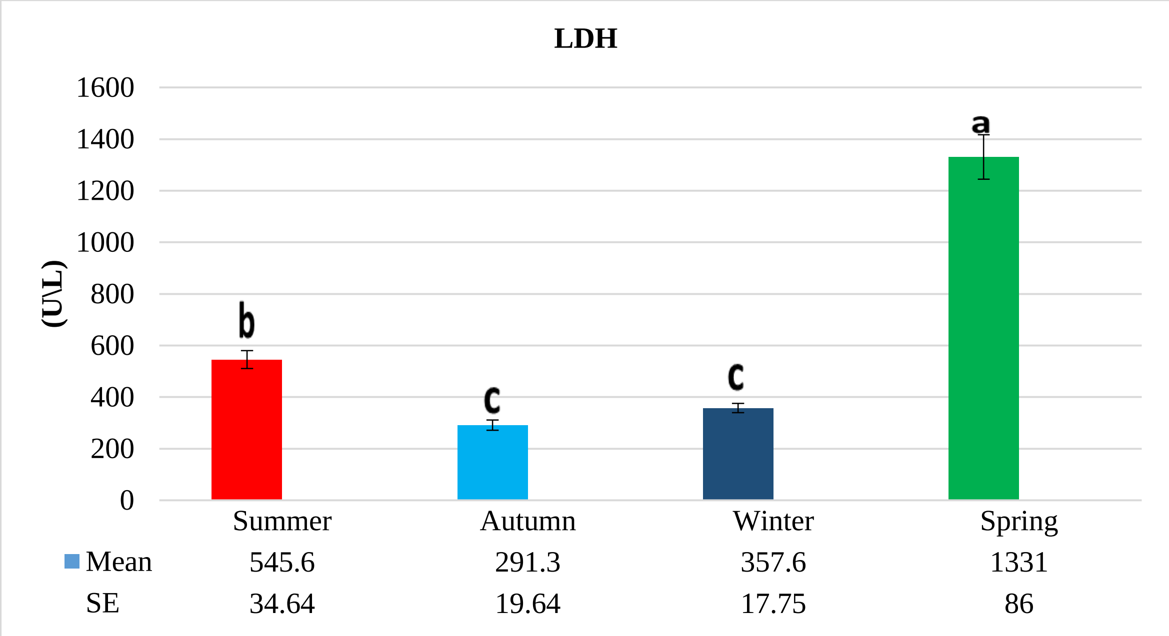

Figure. 14. Mean activity ± SE of LDH of *T. zillii* during summer, autumn, winter and spring.

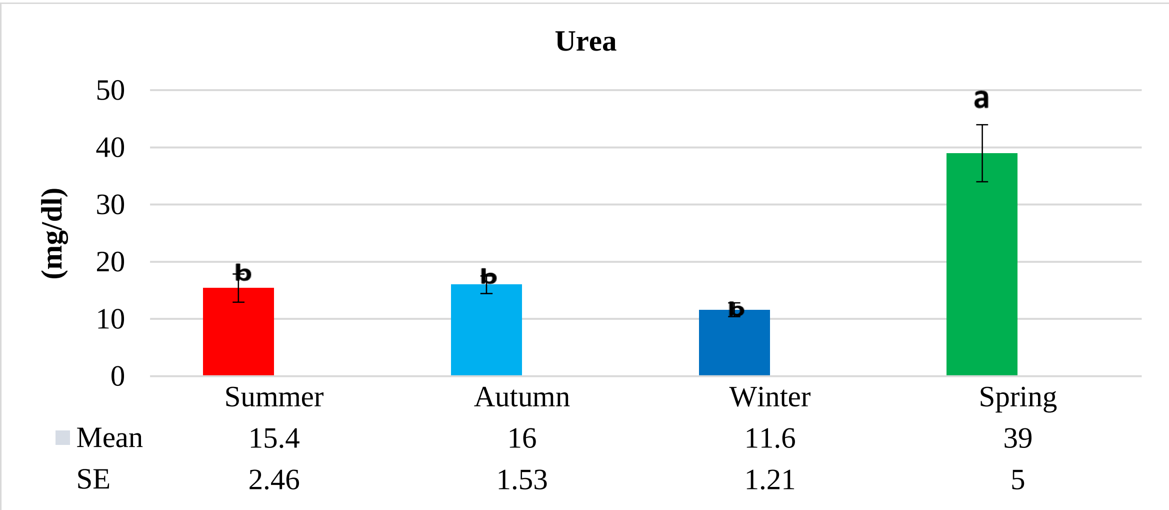

Figure. 15. Mean concentration ± SE of Urea of *T. zillii* during summer, autumn, winter and spring.

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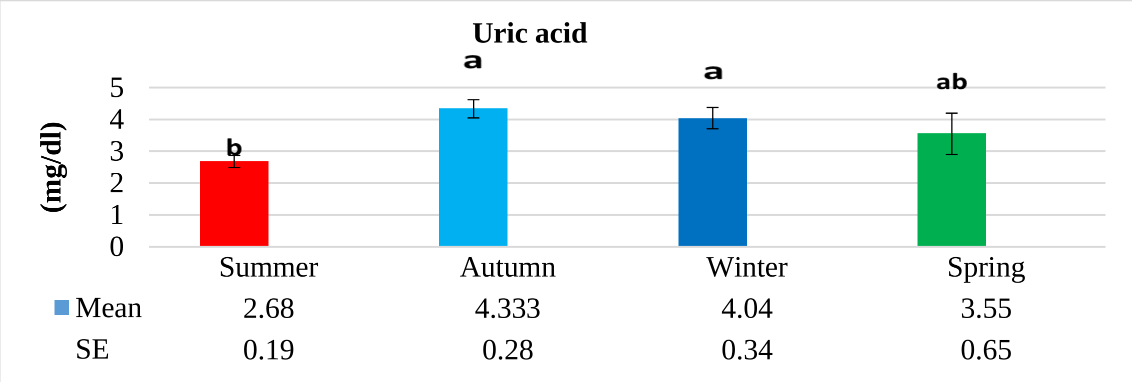

Figure. 16. Mean concentration ± SE of Uric acid of *T. zilii* during summer, autumn, winter and spring.

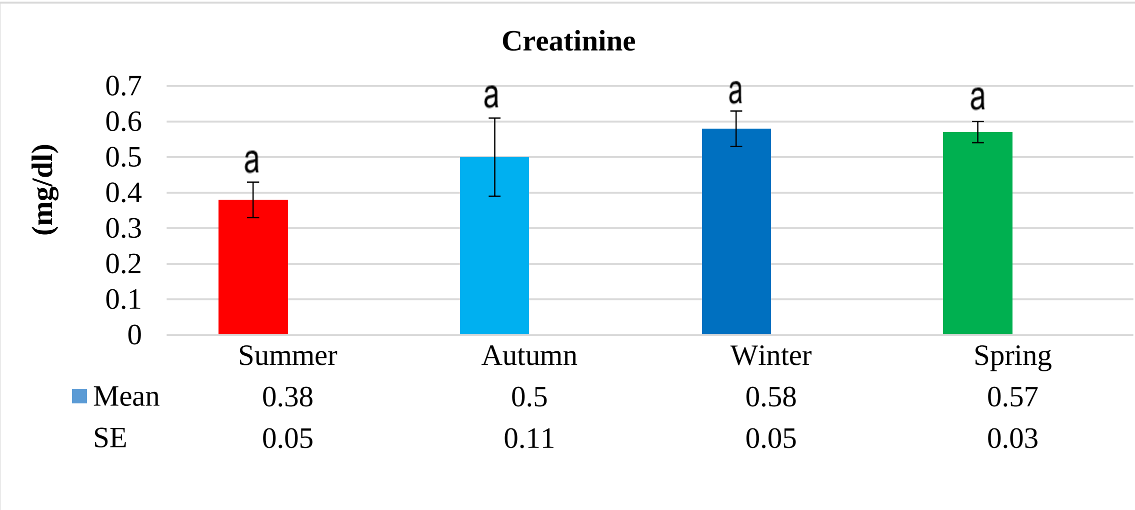

Figure. 17. Mean concentration ± SE of Creatinine of *T. zillii* during summer, autumn, winter, and spring.

Binary correlations between liver and kidney functions parameters

Strong correlations were observed between LDH/Urea (r: 0.871**) and LDH/GPT (r: -0.707**) (Table 4). Moderate correlations were observed for Uric acid/GPT (r: 0.517*) and Uric acid/creatinine (r: 0.517*). All the other binary correlations had low "r" and were not significant.

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Table (4). Pearson's correlations between liver and kidney function parameters of *T. zillii*.

	GOT	GPT	ALP	LDH	Urea	Uric acid
GPT	.177					
ALP	.387	.198				
LDH	-.105	-.707**	-.010			
Urea	-.109	-.376	.123	.871**		
Uric acid	-.172	.517*	-.228	-.226	-.029	
Creatinine	-.274	-.062	-.440	.331	.388	.517*

DISCUSSION

Tilapia can survive a minimum oxygen concentration of 5 mg, the optimal temperature is 29 to 31 ºC, however, the fish can survive temperatures between 8 and 42 °C, but growth rate declines greatly below 20 to 22ºC, the fish may die at below 10ºC or 11ºC (Phillip and Ruwet, 1982; Teichert-Coddington *et al*., 1997; Anene, 2005; Nehemia *et al*., 2012; Mohammed, 2018; Ali, 2019; and Popma and Lovshin, 1995). *T. zillii* tolerates brackish water salinity ranging from10 to 30‰ (El-Sayed, 2006; El-Sawy, 2006; Mohammed, 2018; and Ali, 2019) and a pH of six to nine. The maximum published length of *T. zillii* is 40cm and a maximum weight of 300 grams. All the stated above optimum environmental conditions need for normal growth, reproduction and proliferation of *T. zillii* are available at Ras Al-Tin. However, in winter and summer, ambient temperature may decrease or rise above the stated optima for several days, especially at night and day, likewise, extrema salinity variations may occure during fall and summer. Nevertheless, the fish has adapted itself very well and is now contributing significantly to the artisanal catch. the maximum and mean length of the collected samples were 22.0 and 16.8 cm, the weights were700 227.26gm consecutively.

Fish are very susceptible to physical and chemical changes in their aquatic habitats. Water temperature, salinity, pH, and dissolved oxygen concentration may affect magnitudes of their CBC parameters, and liver and kidney function indicators (Forghaly *et al*., 1973; Wilson and Taylor, 1993; Zohreh *et al*., 2013, Fredianelli *et al*., 2018). In the present study, magnitudes and activities of these parameters did not assume dominant or regular seasonal trends. However, in general, they were relatively lower in summer and winter, and relatively higher in autumn and spring. Although these variations are acknowldgly caused by seasonal changes in the habitat parameters, yet no specific habitat parameter/s could be cited as the causative agent of a given individual hematological change, and hence, hematological changes may be considered normal seasonal variations. Diseased fish were not observed within the studied samples, and the lagoon and the surrounding area is free from pollution. In the present study, WBC was higher in autumn and lower in the rest of the year. Possibly, this resulted from the freshwaters run off from land that drains in the lagoon bringing with it minor contaminants and bacteria that had accumulated in the surrounding land; By advent of winter, most of the contaminants and bacteria that had entered the lagoon were carried away by currents to open sea, causing a decline in WBC. RBC was highest in autumn and lowest in summer. MCV was not affected by seasons. Hb was highest in winter and autumn and lowest in summer. Saleh (1999) stated that theoretically, RBC count and Hb concentration is known to have inverse relationship with

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availability of oxygen in the habitat, however, this was not the case in the present study. MCH and MCHC winter maximum was significantly different from summer, autumn and spring values, a trend somewhat similar to that of RBC and Hb. Changes in PLT values throughout the four seasons were not significant. Bhat, (2017) in his study on *Schizothorax niger* found that the highest number of leukocytes was found in spring and the lowest number in winter. Hemoglobin and Hematocrit values were highest during the months of summer and lowest during winter. In the present study, GOT and GPT were highest in autumn, ALP was highest in autumn and summer. GOT, GP T and ALP are liver function indicators enzymes; an increase in their activity reflects increased liver activity, their magnitudes suggest increased liver activity in autumn. This is plausible because in autumn, the land runoff brings with it terrestrial contaminants and bacteria to the habitat of the fish. Liver is the main organ that is concerned with detoxification of such toxins. Fredianelli *et al* (2018) reported that, an increase in the rate of ALP activity in the autumn season compared to other seasons, as well as with a study conducted on species of the Cyprinids family. LDH (Lactate dehydrogenase) is an enzyme found in living cells where it converts lactate to pyruvate and back (Saleh, 1999). Because it is released in the blood during tissue damage, it is a marker of common injuries and disease. LDH was highest in spring, and then summer, it waa lowest autumn and winter. In the marine environment, spring, and to some extent summer, are seasons of intense activity (following the winter dormancy) of marine organisms; phytoplankton blooming and consequent zooplankton proliferation take place during these seasons (Saleh, 1999); many coastal animals spawn during spring and summer so that their larvae will find enough food and moderate ambient temperature when they hatch. The reproductive season of *T. zillii* in Oued Righ wetland in Southeast Algeria was from April to July (Zouakh *et al*., 2016). In Abu Qir Bay, Egypt, the spawning season of *T. zillii* was from June to September (Akel and Moharram, 2007), but Mustafa (2014) reported May to August. Based on these information, one can conclude that the spawning season of *T. zllii* in Ras Al-Tin is also during spring and summer, and that the high LDH values during these seasons resulted from the increased activities that followed the winter dormancy. In vertebrates, the final product of protein catabolism is ammonia, but ammonia is toxic, and so is converted to urea and uric acid as soon as it is formed, urea and uric acid are excreted with urine. In the present study, urea concentration was highest in spring. Uric acid concentration was maximum in autumn, winter and spring and minimum in summer. Possibly these seasonal changes were caused by the high spring catabolism that resulted from the increased activity and anabolism during this season. Creatinine is found in vertebrates where it facilitates recycling of adenosine triphosphate (ADP/ATP/ADP), the energy currency of the cell, primarily in muscle and brain tissue (Saleh, 1999). Creatinine also acts as a buffer. In the present study, creatinine concentration was constant throughout the year, seasonal differences were insignificant. This may be because the body had the ability to store excess creatinine in its muscle tissue so as to spend it in seasons in which it encounters inadequate creatinine levels (Volek *et al*., 2008) therefore, excess and deficiency of creatinine that may show up in muscle did not show up in blood.

CONCLUSION

Tilapia. Zillii was introduced in Ras Al-Tin lagoon in the early 1990ties, since then. it has adapted itself very well to its new habitat, and is now an important component of the artisanal catch. Its hematological parameters (CBC and liver and kidney functions) were normal.

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Implication to research and practice

Tilapia zillii is a sturdy culture fish that can adapt very well to new habitat in a relatively few years.

Future research

There are some preliminary evidences that *Tilapia zillii* can adapt, over years, to environmental conditions outside the ranges stated in previous literature. We suggest conducting a comparative study on the ability of this fish to survive in environments integrated over a large geographical gradient, based on secondary sources derived from published studies,

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