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Article

Effect of Seasonal Changes on Physiological and Histological Characteristics of Nile tilapia (*Oreochromis niloticus*) Inhabited Two Different Freshwater Habitats

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Abstract: The present investigation aimed to assess the effect of seasonal changes on physiological and histological characteristics of Nile tilapia (*Oreochromis niloticus*) inhabited two different freshwater habitats (Damietta branch of Nile River and Ammar Drain) over the period from March 2015 to February 2016. Samples of fish blood and tissues were seasonally collected and somatic indices, hematological parameters (Hb, RBCs, Ht, WBCs), oxidative stress biomarkers (SOD, CAT), and histopathological changes (in skin, muscles and gonads) were investigated. Studied parameters showed significant seasonal changes in investigated localities. However, their levels were higher in Ammar Drain than those of other site. Biomarkers revealed that water contaminants led to negative impacts on the physical status, growth indicators, hematological parameters, enzyme activity, and histological features of fish. Nile tilapia showed varying physiological responses in both studied ecosystems. The hematological and biochemical portrait of fish blood indicated proper physiological adaptations to the polluted water of Ammar Drain. The vast majority of the hematological parameters recorded lower levels in Ammar Drain than in River Nile. There were significant increases in WBC counts in Nile tilapia inhabiting Ammar Drain than those of River Nile. This may reflect the immunological challenge facing fish in such deteriorating environment. The present investigation indicated overall lower plasmatic CAT and SOD activities of Nile tilapia from Ammar Drain compared to those sampled from River Nile. Different water pollutants are the likely major source of the physiological anomalies in fish. Accordingly, the decreased enzyme activities in fish inhabiting Ammar Drain seems likely to indicate an insufficient response to overcome the prominent oxidative stress.

Moreover, plasmatic CAT and SOD activities of Nile tilapia attained clear seasonal variation in the two investigated ecosystems. The fish sampled from Ammar Drain exhibited some histopathological signs showing alterations in the skin, muscles and oogenesis or spermatogenesis of Nile tilapia

Keywords: Nile tilapia, Water pollution, Ammar Drain, River Nile, Hematology, Oxidative stress, Histopathology.

1. Introduction

Fish as well as other aquatic organisms are ideal models for the evaluation of bioindicators of pollution-induced oxidative stress. Ben Ameur *et al.* (2012) suggested that fish play a two-fold function of being on the uppermost level of the food chain and react powerfully to environmental stress. Powerful biomarkers are utilized to decide if there is evidence of substantial pollutants introductions that have surpassed detoxification processes and led to negative impacts on the physiology and biochemistry of the living organism (Vijayavel *et al.,* 2004). According to Doherty *et al.* (2010), cellular bioindicators function as an early analyzer because they can reveal differences at the level of the cells and molecules before developing at higher biological organization ranks.

Seasonal fluctuations of the biological adjustments include biological parameters such as reproduction and metabolism of fish, and environmental parameters such as food accessibility, dissolved oxygen, water temperature, salinity, photoperiod, etc. (Parihar *et al.*, 1997 & Buet *et al.,* 2006). Thus, these seasonal changes which are common in field studies based on bioindicator may alter the significance of biomarker system (Da Rocha *et al.,* 2009). Depending on the allocation of antioxidant resistances in various tissues, living organisms exhibit varying antioxidant responses against environmental stressors. Fish responses to environmental stressors can be correlated with the anatomical positioning, route of exposure and distribution pattern of contaminants, in addition to defense ability (Ferreira *et al.*, 2005 & Ahmad *et al.*, 2006 & Ruas *et al.,* 2007 & Da Rocha *et al.,* 2009). Fish represent one of the most nutritive and cheap sources of the animal protein. Fish contribute to about 6% of the world supply of protein and about 24% of the animal protein (El-Badry, 2010 & Mashaly, 2011). Nile tilapia, *Oreochromis niloticus* (L.) is a fish of economic importance in tropical and subtropical countries and it highly consumed by a large sector of Egyptians. This fish is more abundant throughout the year than many other Egyptian fish. It is an important and cheap source of the animal protein (Kime *et al.*, 1996 & El-Badry, 2010). A promising development program of fish resources could solve the problem of animal protein deficiency at qualitative and quantitative levels (Nguyen, 2009). In this respect, the

proper investigation of different aspects of the fish biology and ecology are strongly recommended (Abdel-Meguid, 1989 & El-Etreby *et al.*, 1993).

There is growing evidence that hematological and biochemical variables can offer adequate data of fish undergoing pollution problems (Cazenave, 2005 & Li, 2011& Gaber *et al.,* 2013). The direct relation between the circulating blood and surrounding environmental fluctuations, and the availability of fish blood are regarded as important biomarkers (Cazenave, 2005 & Li, 2011 & Gaber *et al.*, 2013). The response of the fish to environmental stressors could be monitored by an array of bioindicators of the oxidative stress (Achuba and Osakwe, 2003 & Farombi *et al.,* 2007 & Monterio *et al.,* 2007 & Pavlovi *et al.,* 2010 & Doherty *et al.,* 2010). The present study aimed at evaluating effect seasonal changes on physiological and histological characteristics of Nile tilapia (*Oreochromis niloticus*) in two different freshwater habitats, Egypt.

2. Materials and Methods

2.1. Study Area

The present study was conducted during the period from March 2015 to February 2016. Two different aquatic ecosystems were selected. This study was performed according to the protocol designed by El-Naggar *et al.* (2016): Ammar Drain (Drain No. 2) at Belqas city as a polluted environment and Damietta Branch of River Nile at Al-Tawailah village as a reference site 50 km north Mansoura City and has the following coordinates: 31°22'46.4556" N 31°29'13.2432" E (Figure 1).

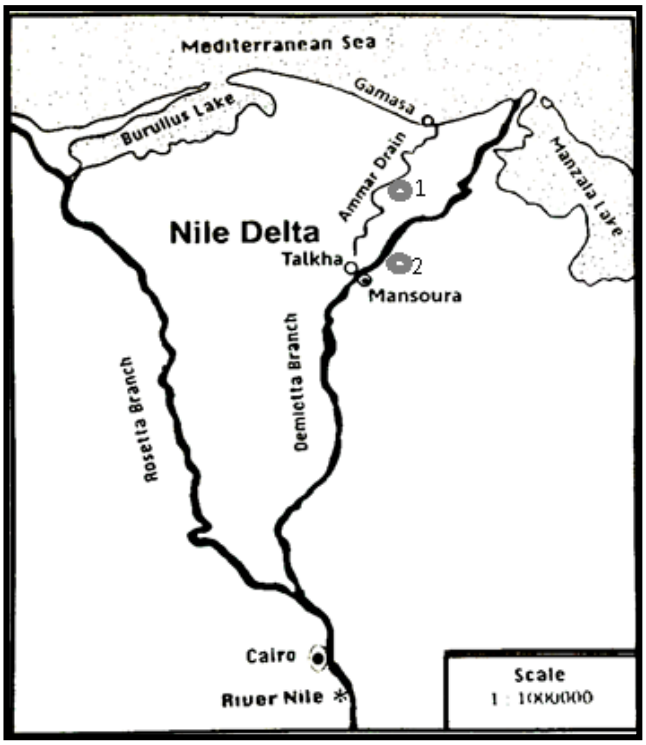

Figure 1: Map showing the study areas: Ammar Drain (1) and the River Nile (2)

2.2. Collection and Analysis of Fish Samples

About 20-30 samples of Nile tilapia, *O. niloticus* were collected seasonally from each sampling site from River Nile and Ammar Drain. Fish samples were transported alive in aerated tanks with plentiful amount of water to the laboratory for different investigations. Fish were placed in large plastic containers accommodating natural water to minimize stresses and injuries. Records were kept of the total body length, weight and sex. Moreover, the gonads were weighed.

2.2.1. Hematological and biochemical analysis

Before fish dissection, blood samples were obtained from the caudal vein by a hypodermic syringe. Then, each sample was immediately divided into two portions; the first one used EDTA as an anticoagulant for measuring complete blood counts, while the second one was centrifuged at 3000 rpm for 10 minutes without anticoagulant. The collected sera were kept at -20 °C till analysis.

The whole blood was immediately used for the estimation of RBCs and total WBCs counts under the light microscope using Brand count chamber (hemocytometer) following dilution of the blood in phosphate buffer at pH of 7.2 (Dacie and Lewis, 1984). Hemoglobin (Hb) content was determined colourimetrically by measuring the forming of cyanomethaemoglobin according to Van Kampen and Zijlstra (1961). Haematocrit (Ht) ratio was directly estimated after blood collection, by transferring a small amount of blood in to a capillary tube and centrifuging them for 5 min in an ABX Micros 60 device, manufactured by HORIBA ABX SAS.

Levels of superoxide dismutase (SOD) enzyme in serum were estimated with the aid of Cayman Kit (Biodiagnostic Company, Mansoura, Egypt), according to the method described by Nishikimi *et al.* (1972). Serum levels of catalase (CAT) activity were determined using Cayman Kit (Biodiagnostic Company, Mansoura, Egypt), according to the procedure estimated by Aebi (1984).

2.2.2. Histopathological manifestations

Following dissection, appropriate segments of fish muscles, gonads (testes and ovaries), and skin were preserved in 10% formaldehyde and processed for embedding paraffin, then sectioned at 5 μm thickness and stained in hematoxylin and eosin according to Roberts (2012). Histological investigations were studied with the aid high power Leitz Labor Lux light microscope and the imperative histological features were captured by a digital camera connected to the microscope.

2.3. Statistical Analysis

All values are given as (Mean ± SD). On the other hand, the seasonal differences of the physicochemical parameters, blood parameters and water quality indices in each locality were tested

using one-way ANOVA. Tukey HSD was used as a post hoc test to detect significant differences between seasons at $P < 0.05$. All the statistics were done by using SPSS package (version 20 for Windows).

3. Results

3.1. Hematological Parameters

The seasonal values of Hb content in `sampled from both sites are recorded in Table 1. It can be seen that highest Hb value (11.43 g/dl) was estimated during winter at River Nile samples, while lowest value (7.47 g/dl) was obtained during autumn in Ammar Drain samples. A highly significant difference of the Hb content in Nile tilapia was recorded between River Nile and Ammar Drain ($t= 3.231$, $p \leq 0.01$) but, there was no significant seasonal variation in Hb values in Ammar Drain samples ($F= 1.26$, $p > 0.05$). Tukey HSD test indicated significant difference of this blood parameter of Nile tilapia in River Nile between autumn and winter.

The seasonal fluctuations of RBCs collected from Nile tilapia sampled from both sites are recorded in Table 1. The highest RBCs count (2.34 10^6/μL) was estimated during summer in River Nile while, the lowest value (1.46 10^6/μL) was recorded during winter in Ammar Drain. The RBCs count of Nile tilapia showed no significant changes between the two study areas (t-test: $t= 1.645$, $p > 0.05$) or among seasons ($F= 1.121$, $p > 0.05$).

Regarding Hct levels, its highest value (32.13 %) was estimated during summer in River Nile, while its lowest value (24.67 %) was recorded during winter in Ammar Drain with no significant difference between both sites ($t=0.804$, $p > 0.05$) or among seasons ($F= 0.929$, $p > 0.05$) (Table 1)

The highest WBCs count (210.57 x 10^3/μL) of Nile tilapia was found during spring in Ammar Drain, while the lowest value (126.27 x 10^3/μL) was recorded during winter in River Nile (Table 1). A significant difference in WBCs count of Nile tilapia was observed between the two investigated freshwater environments ($t= -2.504$, $p \leq 0.05$). Further statistical analysis (Tukey HSD test) detected significant differences for this parameter in River Nile between winter and other seasons. In contrast, no significant seasonal changes were recorded in WBCs count at Ammar Drain ($F= 0.256$, $p > 0.05$).

Table 2shows the seasonal values of SOD enzyme in the blood collected from Nile tilapia inhabiting River Nile and Ammar Drain. It is obvious that highest SOD value (337.37 u/ml) was estimated during winter in Ammar Drain, while lowest value (126.73 u/ml) was recorded during autumn in Ammar Drain with no significant changes between River Nile and Ammar Drain ($t= 1.005$, $p > 0.05$). The seasonal fluctuations of SOD activity in Nile tilapia at River Nile were insignificant statistically ($F= 2.243$, $p > 0.05$), while highly significant seasonal changes were recorded at Ammar Drain ($F= 13.209$, $p \leq 0.01$). Tukey HSD recorded significant differences in SOD activity at Ammar Drain between summer and winter as well as autumn and winter.

Table 1. Seasonal changes in the blood parameters (Mean ± SD) of Nile tilapia, *O. niloticus* sampled from River Nile and Ammar Drain over the period from March 2015 to February 2016.

Blood / Season	River Nile				Ammar Drain			
	Hb (g/dl)	**RBCs ($\times10^6$/µL)**	**Hct (%)**	**WBCs ($\times10^3$/µL)**	**Hb (g/dl)**	**RBCs ($\times10^6$/µL)**	**Hct (%)**	**WBCs ($\times10^3$/µL)**
Spring	9.87 ± 0.51	1.61 ±0.07	27.40 ±1.23	191.00 ±10.95	8.07 ± 0.45	1.60 ±0.02	25.87 ±1.06	210.57 ±4.67
Summer	9.80 ±0.10	2.34 ±0.15	32.13 ±2.23	201.23 ±6.05	7.80 ±2.86	1.84 ±0.45	30.27 ±6.70	201.63 ±13.68
Autumn	8.27 ± 1.29	2.09 ±0.62	30.07 ±4.39	192.10 ±12.44	7.47 ±1.19	1.81 ±0.30	29.47 ±5.35	201.87 ±20.80
Winter	11.43 ±0.51	1.66 ±0.24	26.11 ±1.36	126.27 ±2.65	8.33 ±1.82	1.46 ±0.27	24.67 ±4.58	199.33 ±22.50

Hb = Hemoglobin RBCs = Red blood cells Hct = Hematocrit WBCs = White blood cells

Table 2. Seasonal changes of serum superoxide dismutase (SOD) and catalase (CAT) enzymes (Mean ± SD) of Nile tilapia, *O. niloticus* sampled from River Nile and Ammar Drain over the period from March 2015 to February 2016.

Serum / Season	River Nile		Ammar Drain	
	SOD (u/ml)	**CAT (u/l)**	**SOD (u/ml)**	**CAT (u/l)**
Spring	175.00 ±78.06	869.10 ±137.04	225.00 ±75.00	605.00 ±180.90
Summer	249.93 ±62.45	750.13 ±192.20	145.77 ±36.14	472.43 ±111.60
Autumn	246.08 ±34.18	917.16 ±80.28	126.73 ±35.20	669.31 ±147.13
Winter	299.87 ±54.37	324.77 ±391.15	337.37 ±12.56	231.91 ±125.36

SOD = Superoxide dismutase enzyme CAT = Catalase enzyme

The seasonal values of CAT enzyme in the blood collected from Nile tilapia inhabiting River Nile and Ammar Drain are shown in Table 2. It can be noticed that highest CAT value (917.16 u/l) was

estimated during autumn in River Nile, while lowest value (231.91 u/l) was recorded during winter in Ammar Drain with a significant difference between River Nile and Ammar Drain ($t= 2.186$, $P \leq 0.05$). Additionally, Tukey HSD test detected a significant variation in CAT activity of Nile tilapia from River Nile between autumn and winter ($F= 4.055$, $p \leq 0.05$). The corresponding values of CAT activity in Ammar Drain were significant between spring and winter as well as autumn and winter ($F= 5.438$, $P \leq 0.05$).

3.2. Histopathological Examination

3.2.1. Sections of fish skin

The skin of Nile tilapia is hard, irregular and comprises two main layers: an outer epidermis and an inner dermis (Figure 2 A and B). A basement membrane is found between the epidermis and dermis. The epidermis is multilayered and thinner than the dermis. It is formed of flattened epithelial cells (stratified epithelium) and has no blood vessels. The dermis is composed mainly of tight fibrous connective tissue. It comprises a thin upper layer (loose connective tissue) and a thick dense layer (stratum compactum), formed of fibrous connective tissues. Some blood vessels are recognized in the dermal layer. Closely below the dermis, and separating the dermis from the body musculature, a layer of loose connective tissue called the hypodermis or subcutis is observed.

Many types of gland cells could be seen among the constituents of the epidermis (Figure 3 A, B, C and D). Moreover, the epidermis is loosened and showed signs of degeneration. The epidermal architecture is altered and many polygonal and cystic gland cells are amorphous. Lymphocytic infiltration is relevant in Figure 3 (C and D). The skin of Nile tilapia inhabiting Ammar Drain showed irregular skin surface, vacuolation and degenerating tissue matrix, loss of tissue architecture compared to the skin of their conspecifics from Rive Nile (Figure 2 B).

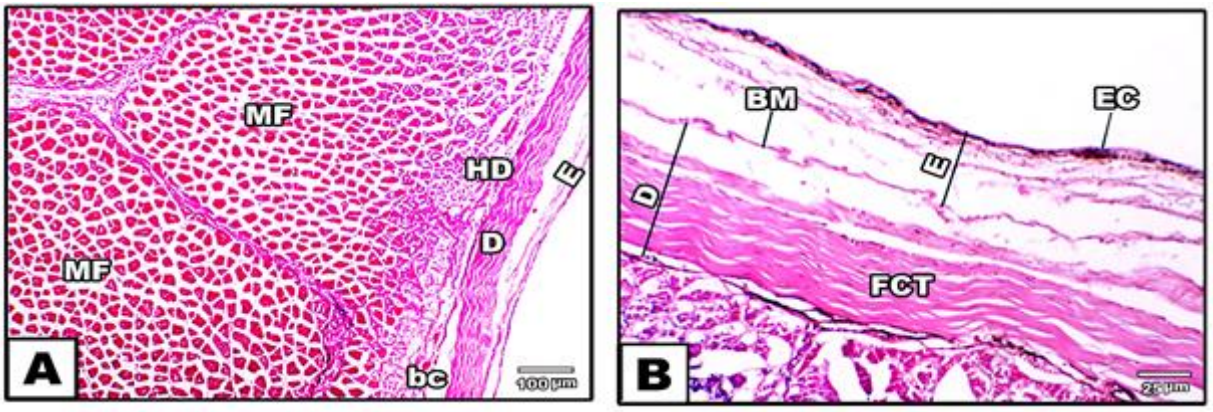

Figure 2. Photomicrograph showing the normal histological features of the skin of Nile tilapia (A and B) inhabiting River Nile. Scale bar = 100, 25 µm. BC, blood cell; BM, basement membrane; D, dermis; E, epidermis; EC, epithelial cell; FCT, fibrous connective tissue; HD, hypodermis; MF, muscle fiber. **(H&E x100, x400)**

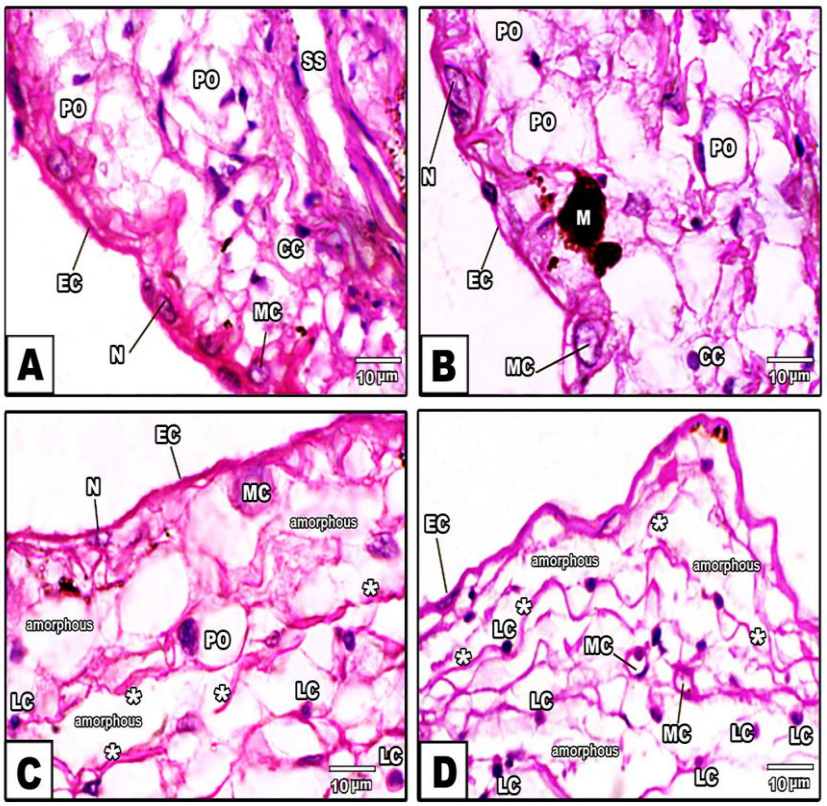

Figure 3. Photomicrograph showing vertical section of the normal histological features of the skin of Nile tilapia (A and B) inhabiting River Nile in comparison with the corresponding histopathological changes of the skin of Nile tilapia (C and D) inhabiting Ammar Drain. Scale bar = 10 µm. CC, cystic cell; EC, epithelial cell; LC, lymphocytic infiltration; M, melanocyte; MC, mucous-producing cell; N, nucleus; PO, polygonal cell; SS, scale sac; asterisks, fungal infection. Note that the epidermis of Nile tilapia from Ammar Drain shows lymphocytic infiltration and becomes amorphous. **(H&E X 1000)**

3.2.2. Sections of fish muscles

As seen in Figure 4 (A and B), muscles of Nile tilapia is well–organized and is formed of closely packed muscle bundles. The muscles bundle is polygonal in shape and possesses a variable number of marginal or peripheral nuclei. On the other hand, muscles of Nile tilapia from Ammar Drain had loosened and widely–spaced muscles fibers during winter and summer, respectively. Each muscle bundle is irregular in shape and possesses peripheral nuclei and encysted parasitic from is enveloped by a loosened fibrous tissue.

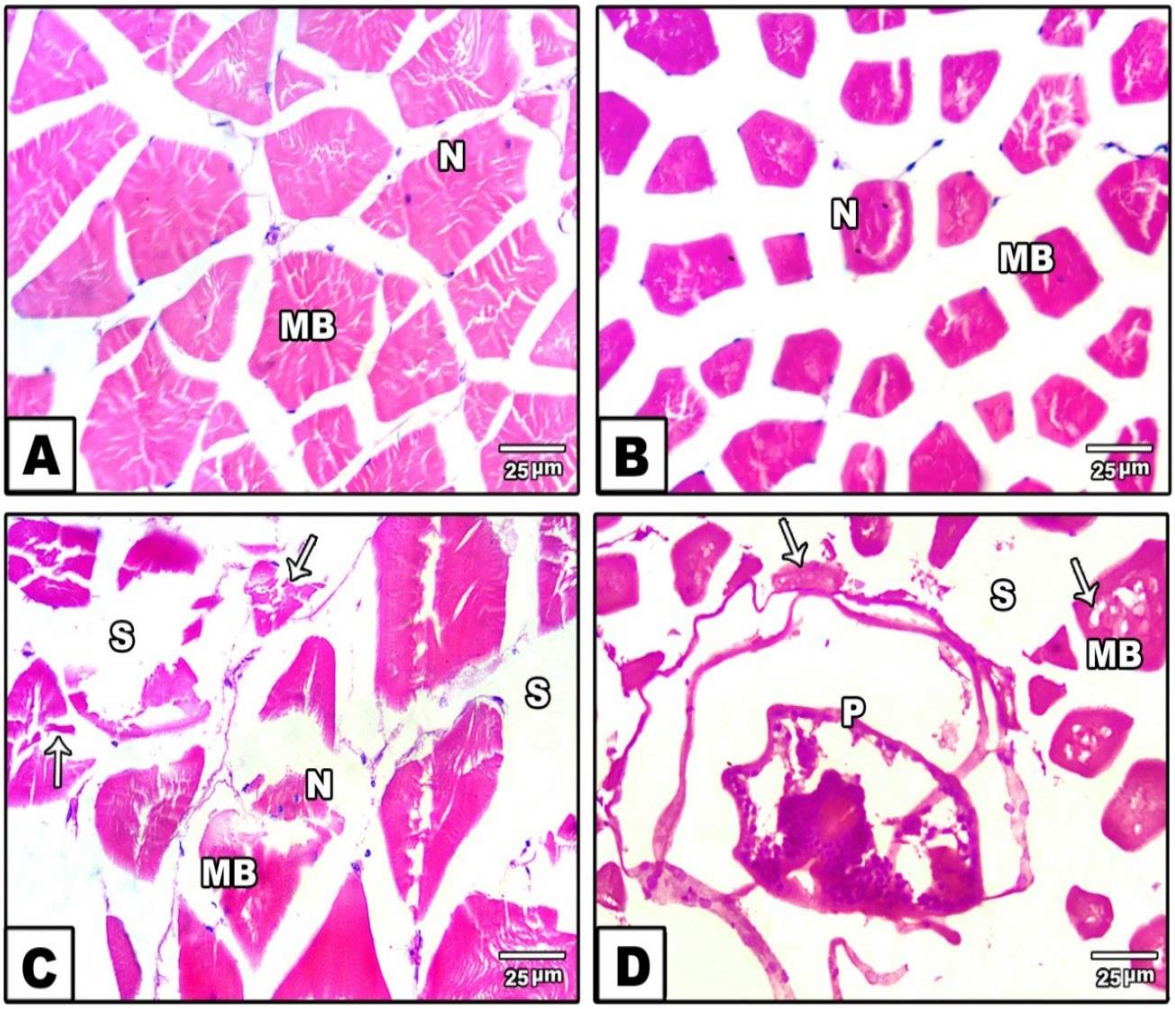

Figure 4. Photomicrograph showing the histological features of the muscle fibers of Nile tilapia dwelling River Nile (A and B) and Ammar Drain (C and D), during winter and summer, respectively. Scale bar = 25 µm. MB, muscles bundles; S, spaces between muscle bundles; N, nucleus of the muscle bundle; P, parasitic cyst; arrow, disintegrated fibers **(H&E X 400)**.

3.2.3. Sections of fish ovary

Regarding the normal ovarian histology of Nile tilapia sampled from River Nile, full–developed oocytes, loaded with vitellioe cells are evident. Also, previtellogenic and postvitellogenic forms are apparent in (Figure 5 A and B). The postvitellogenic form is more or less circular in shape and possesses a central, round nucleus which occupies the majority of the cytoplasm. Concerning the abnormal ovarian histology of Nile tilapia inhabiting Ammar Drain, a marked degradation and resorption of oocytes, degeneration and vacuolation of the cytoplasmic matrix, disordered oogenesis and collapse of the germinal epithelium are evident. During summer months, the degenerative effect of pollution on the gonadal development of Nile tilapia was more intense. The stages of the gonadal development are incomplete and deformed. Marked vacuolation could be noticed throughout the oocyte. Moreover, there is a fewer number of vitelline cells.

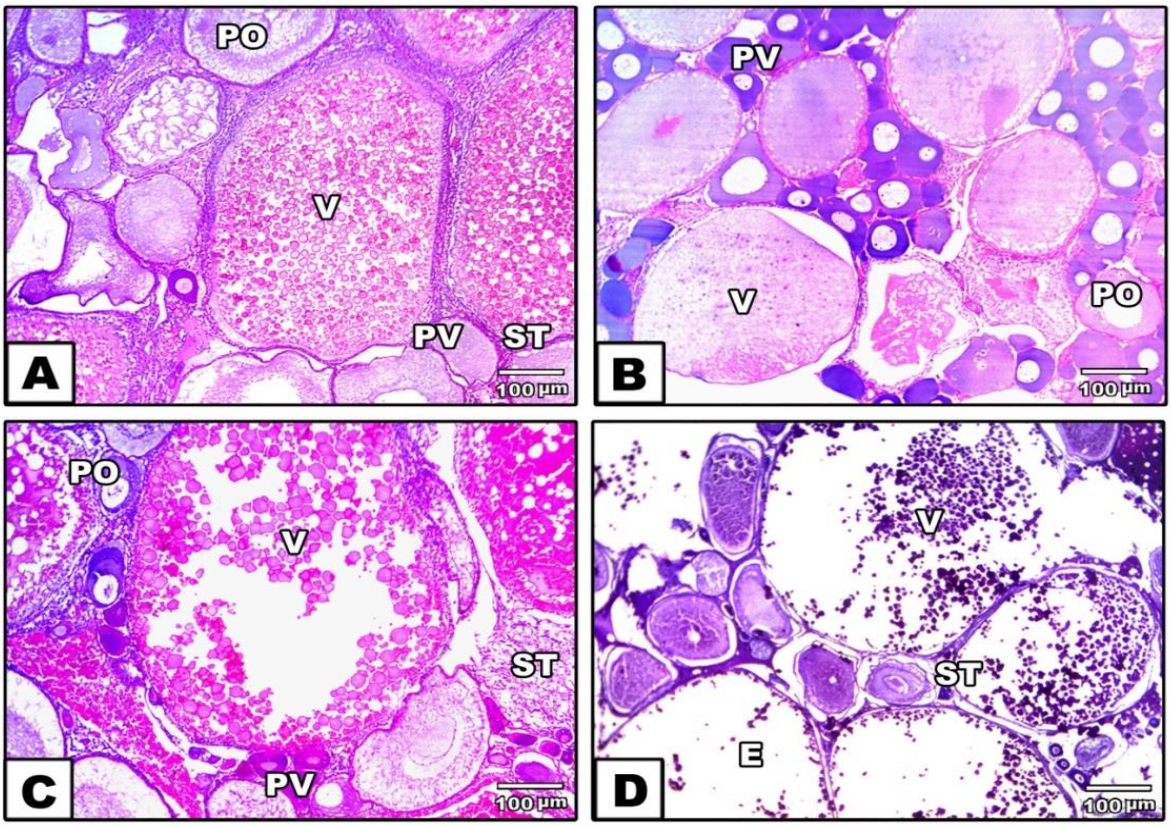

Figure 5. Photomicrograph showing the histological features of the ovary of Nile tilapia from River Nile during winter (A) and summer (B), and the histopathological features of the some fish from Ammar Drain during winter (C) and summer (D). Scale bar = 100 µm. E, empty follicle; PO, postvitellogenic stage; PV, previtellogenic stages; ST, stroma; V, vitellogenic stages. **(H&E X 100)**

3.2.4. Sections of fish testis

Concerning the presumably normal testis (Figure 6 A and B), well-developed testicular lobules contain the spermatozoa which are centrally located. The testicular lobules are nourished by blood vessels. Regarding altered testis from Ammar Drain, the productivity of the testis is diminished and the testicular lobules lose most of the spermatogenic cells. As shown in Figure 6 (C and D), spermatogenesis is incomplete where many stages are absent, and spermatozoa appear scattered in the testicular lobules. Comparing to winter, testis preparations in summer underwent less environmental stress. Some testicular lobules appeared completely empty from spermatozoa.

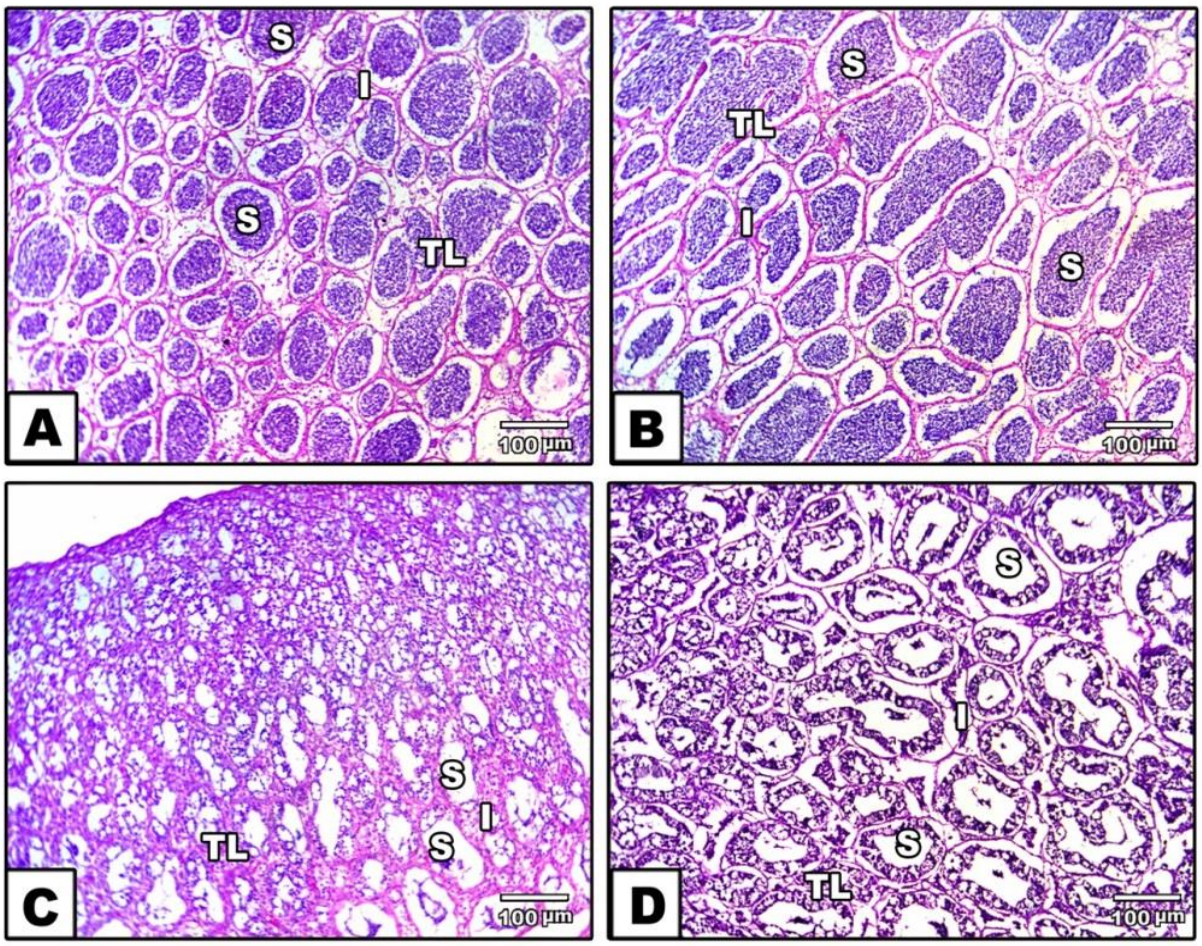

Figure 6. Photomicrograph showing the histological features of the testis of Nile tilapia from River Nile during winter (A) and summer (B), and the histopathological features of the same fish from Ammar Drain during winter (C) and summer (D). Scale bar = 100 µm. TL, testicular lobule; S, spermatozoa; I, interstitium. **(H&E X 100)**

4. Discussion

Blood is a pathophysiological mirror of the internal environment of the living organism and is regarded as a decisive diagnostic tool in environmental pollution studies (Gaber *et al.*, 2013). Exposure to interactive effects of the multisource pollutants leads to negative impacts on the hematological indices (Vutukuru, 2005). The present study showed that all hematological parameters of Nile tilapia sampled from Ammar Drain were lower than those sampled from River Nile. This could be a reflection of fish response to environmental pollution (El-Naggar *et al.*, 2016 & Tayel *et al.*, 2007). The decreases of these parameters goes in line with elevation in the levels of chemical parameters studied such as nitrogen and phosphorous, DO depletion and the increase in TOC as a result of pollution stress in that area (Tayel *et al.*, 2007).

There was a positive correlation between DO and RBCs, Hb, Hct values in Nile tilapia. Under oxygen depletion condition, the liver probably revives erythropoiesis to recompense the desire by elevated oxygen transportation to marginal tissues (Rifkind *et al.,* 1980). Chowdhury *et al.* (2004) recorded a drop of blood Hb and Hct during environmental oxygen depletion and acute exposure to water pollutants to diminish the blood oxygen and carrying capacity following impairment of the gas exchange. Singh (2008) suggested that different water pollutants are the possible chief source of the physiological disorders of fish. The decrease in RBCs, Hb and Hct values in fish dwelling Ammar drain may be related to water pollution.

The WBCs are the regulator of the immune system; its count was elevated in the blood of Nile tilapia sampled from Ammar drain. This could be attributed to the exposure to chronic sewage and domestic discharges, and agricultural wastes. This is in agreement with the results of Gaber *et al.* (2013) who suggested that the increase of WBCs in the blood of catfish caught from El-Rahawy Drain could be a result of exposure to chronic sewage. The increase of WBCs count may be attributed to a general immune response to pollution (Nussey *et al.,* 2002). According to Dick and Dixon (1985), increased WBCs count in fish subjected to pollutants indicates leukocytosis or an increase in the total WBCs value over the normal range. Stimulation of lymphopoiesis (i.e. generation of lymphocytes) and/or intensify or magnify the production and discharge of lymphocytes from the lymphomyeloid cells in response to the stress induced by toxic substances may lead to an elevation in WBCs count (El-Sayed *et al.,* 2007). The fish from River Nile was found to be healthier compared with those from Ammar Drain. Fish sampled from Ammar drain seemed darker and unhealthy in appearance. This might be due to the high pollution level in Ammar Drain compared to River Nile.

Oxidative stress biomarkers such as SOD and CAT enzymes are commonly employed for environmental hazard impacts assessment (Livingstone, 2001 & Tsangaris *et al.,* 2011). The present work revealed lower SOD and CAT activities in the blood of Nile tilapia sampled from Ammar Drain compared to those from River Nile. The changes of Oxidative stress biomarkers in Ammar drain can be interpreted due to water pollution. Different water pollutants are the possible main source of the physiological disorders in fish (Adams *et al.,* 2001 & Viarengo *et al.,* 2007). Previous studies on grey mullet, Mugil cephalus and sardine, Sardina pilachardus indicated higher SOD and CAT levels in fish inhabiting stressed ecosystems (Rodriguez-Ariza *et al.,* 1993 & Peters *et al.,* 1994). Activation of oxidative stress enzymes can be motivated by production of reactive oxygen species (ROS) as a protection mechanism against oxidative stress (Winston and Di Giulio 1991) or inhibited due to toxicity that impedes the ROS creation (Cossu *et al.,* 1997). The enzyme response to pollutants displays an early elevation in activity as a result of enzyme induction, followed by a decline in action as a result of improved catabolic pathways and/or inhibition by contaminants (Viarengo *et al.,* 2007).

In the present investigation, decreased oxidative stress enzymes activity in fish sampled from Ammar Drain can be correlated to the deficiency to recompense for imbalance of the release of free radicals and oxidative defense mechanisms, probably a consequence of intense pollutant gradient. Concerning oxidative stress enzymes, the principal protection against undesirable increased oxygen levels comprises SOD enzyme, which stimulates the change of superoxide anion to oxygen and hydrogen peroxide, and CAT enzyme that additionally dissociates hydrogen peroxide to water. Owing to the present findings, the levels of SOD and CAT activities were lower in fish sampled from Ammar Drain than those of River Nile. The drop in those enzyme activities may be correlated to long-term exposure of fish to environmental contaminant. Similar response patterns were documented in previous studies (e.g. Bainy *et al.,* 1996 & Lenartova *et al.,* 1997). Previous studies in sea bass, Dicentrarchus labrax and grey mullet, M. cephalus revealed low SOD and CAT actions in fish dwelling polluted water (Ben Ameur *at el.,* 2012).

There was seasonal variation in SOD and CAT activities of Nile tilapia either in River Nile or Ammar Drain. The obtained results showed that SOD activity increased in spring and winter in Nile tilapia sampled from Ammar drain (the contaminated area) compared to the corresponding values in River Nile. Elevated SOD activity could be attributed to the adverse impacts of pollution on fish (Jordanoska *et al.,* 2008). Activity of CAT enzyme decreased in Nile tilapia sampled from Ammar Drain compared with fish sampled from River Nile. The changes of biochemical parameters in the blood of Nile tilapia sampled from Ammar Drain can be induced by water pollution. Different water pollutants are the possible main cause of physiological anomalies in fish (Adams *et al.,* 2001& Viarengo *et al.,* 2007). This could be an evidence for the response of fish to environmental pollution (Tayel *et al.,* 2007). The decreases of these parameters goes in line with elevation in the levels of chemical parameters studied such as nitrogen and phosphorous, DO depletion and the increase in TOC as a results of pollution stress in that area (Jordanoska *et al.,* 2008 & Osman *et al.,* 2010 & Ben Ameur *et al.,* 2012).

The present study revealed that fish inhabiting Ammar Drain exhibited some histopathological signs where their muscles bundles were obviously loosened and showed marked spacing. The tissues changes in fish sampled from Ammar Drain can be contributed to water pollution. The current study illustrated those histopathological changes of fish muscles may be due to uptake of pollutants coming from sediment and its surrounding environment (Jeffree *et al.,* 2006). The histological assessment of this study corroborates to the findings on pollutants accumulating in the muscles as a degree of disintegration of the muscles fibers was observed. Thophon *et al.* (2003) and Kaoud and El-Dahshan (2010) found that fish exposed to pollutants may undergo histological alterations in the form of degeneration of muscles bundles with certain focal areas of necrosis. Similar results were described by Kaoud and El-Dahshan (2010) who observed that fish exposed to pollutants show degeneration of muscles bundles with atrophy and splitting of muscles fibers.

Light microscope observations on histological preparations of the skin of Nile tilapia sampled from River Nile and Ammar Drain indicated that water quality has pronounced influences on the infrastructure of the integumentary system. Histopathological features of the skin of Nile tilapia inhabiting Ammar Drain included loosened epidermis and with marked signs of degeneration. Many polygonal and cystic gland cells were amorphous, with no definite form or clear shape. Lymphocytic infiltration was noticeable. The skin is an outer wrapper of fish; hence, it is in-full-force of environmental fluctuations. As a consequence, the skin plays a key role as the first line of defense in physical and chemical means (Vernerey and Barthelat, 2014). Moreover, the skin serves for respiration, excretion and osmoregulation (Vernerey and Barthelat, 2014).

The epidermis is a fragile layer, which is regularly sloughed off and regenerated. The scales covering the skin of Nile tilapia act as an additional, physical barrier separating the skin and underlying tissues from the flowing water currents (Helfman *et al.,* 2009). The dermis lies beneath the epidermis. This layer contains blood vessels, nerves, connective tissues and sense organs. The dermis is well supplied by blood vessels; hence, it also provides nourishment to the epidermis.

In the present study, histopathological changes in testis and ovary of Nile tilapia collected from Ammar Drain may be due to their exposure to sewage, domestic, and agricultural wastes as recorded by Tayel *et al.* (2007). Histological preparations indicated alterations in the oogenesis or spermatogenesis of Nile tilapia exposed to pollutants where the development cycles of gametes were incomplete and some stages were malformed. Testicular inflammation was documented as one of the common responses in both aquatic and terrestrial animals exposed to environmental pollutants (Ruby *et al.,* 1987). Similar histopathological changes (lesions) have been reported in fish exposed to pollutants (Gaber *et al.,* 2013).

In female fish, the mean oocytes number is considered as an important criterion for assessment of the reproductive performance in fish (Gaber, 2000 & Gaber *et al.,* 2013). In the present study, the mean oocytes number of Nile tilapia was affected by the polluted environment. Similar negative impacts have been reported in fish exposed to pollutants (Sioson and Herrera, 1996). Mohamed (2001, 2003) recognized histopathological alterations in fish testis and ovary suggesting that this may reduce the ability of fish to reproduce. It is well known that water pollution has a serious inhibitory effect on fish reproduction (Mohamed, 2001 & 2003) resulting in a decrease in their abundance and, consequently, a decline in fish species diversity. In the present study, pollution effects appeared as disruption in gonadal development. It comes in agreement with other studies for fish inhabiting polluted water (Balch *et al.,* 2000). Also, oogenesis and spermatogenesis were influenced by exposure to sewage effluents (Kiparissis *et al.,* 2003).

5. Conclusion

The present data demonstrated that Ammar Drain is heavily contaminated, and water reclamation of this deteriorated ecosystem is strongly recommended. The bad water quality of Ammar Drain has induced changes or modification in the blood profile and alterations in the muscles, gonads and skin histological features of Nile tilapia, *O. niloticus*. The bad water quality of Ammar Drain may cause massive fish kills and induce chronic health problems such as renal failure and hepatic dysfunction, in addition to its deleterious impacts on the soil. To minimize the negative impacts of these contaminants, establishment of pumping stations and prohibition of disposal of waste materials in the waterways are strongly recommended. Moreover, educational programs should be launched to raise the awareness of the community about thorny environmental issues such as water pollution and related socioeconomic consequences.

Future environmental planning must involve establishing wastewater treatment plants in the vicinity of suburban areas close to Ammar Drain in order to improve the conditions of this annoying water stream.

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