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Some Physiological and Biochemical Studies on Marine Fishes Rearing in Suez Canal Fish Farms

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Abstract: The present work was done to elucidate the impact of water quality in private fish farm in Suez Canal area and agricultural drainage water supply source (sectors A and B), Samples of three different fish species: seabream (*Sparus aurata*), Seabass (*Dicentrarchus labrax*), and Meagre (*Argyrosomus regius*) (sectors C, D and E), on the fish physiology, blood picture, biochemistry and accumulation of heavy metals in flesh and gills of the reared fishes. It was found that, water temperature in the different fish farm sectors around the year were significantly different, however, the pH was slightly basic. In spite of the water dissolved oxygen was around the suitable limits, its minimum value was recorded in the fish farm sector (C). Nonetheless, in this fish farm the maximum levels of salinity and total dissolved salts were recorded. Similarly, the ammonia, nitrite and nitrate concentrations were revealed in this fish farm. The relative dominance of heavy metals in the pond's water of the three investigated fish farm sectors all over the period of the study was Fe, Cu, Pb and Cd. However, heavy metals are highly accumulated in fish flesh of all fish farm sectors than in its water column. The muscle content of the detected heavy metals which accumulate in all examined fish species muscle and gills not exceeded the permitted value suggested by FAO/WHO. Metal pollution index realized its minimum levels in water and fish tissue. It is obvious that, the levels of all heavy metals detected in water samples and *S. aurata*, *D. labrax* and *A. regius* muscle tissues (the edible portion of fish) were under the notified permissible limits. Some of the measured parameters slightly exceeded the permissible limits recommended by regulatory agencies.

Keywords: Heavy metals, Seabream, Seabass, meagre, Suez Canal

INTRODUCTION

Aquaculture remains one of the fastest growing food-producing sectors and acts 46.8 per cent from global fish production (FAO, 2018). Suez Canal is, actually, the lonely canal linking between the Mediterranean Sea and the Red Sea. It is located between Suez and Port Said provinces (lying between longitudes 32°20' and 32°35' E and between latitudes 29° 55' and 31° 15' N with an average length of 170 km along the major axis). Suez Canal is a major maritime route facilitates international trade and economic cooperation between the east and west and as a transport pathway for marine organisms is documented by many authors (Hamed *et al.*, 2012).

Problems of sewage pollution of Suez coastal waters have become a point of local concern in the recent years. Signs of this kind of pollution have already been observed in the last few years along the coastal areas of Suez instead of shores. Mariculture in Egypt is still in its infancy and not as developed as its fresh water aquaculture (Rothius *et al.*, 2013). Marine aquaculture is considered a promising direction towards increasing fish production in Egypt another added value of expansion in mariculture is that it would open new markets for exportation, which will reflect positively on the economy.

There was a national and governmental focus towards the expansion of mariculture and huge projects were established beside the Suez Canal in 2016, in addition to Lake Ghilion project in Kafr El-Sheikh and the East Port Said project for the production of sea bream, sea bass, mullet and shrimp (USDA, 2016). Marine fish production in Egypt contributes to about 70% of the total production of marine fish from North

Africa as most of the North African countries depend on fishery catches rather than aquaculture (Rodger and Davies, 2000).

Marine fish species, such as European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) are also cultured in Egypt. The combined production from both species contributes to about 2.8% of the total fish farm production (GAFRD, 2014). Meagre (*Argyrosomus regius*) production started in 2008 with about 2000 tons then reached 5,884 tonnes in 2014. Aquaculture of meagre is desired due to their fast growth rates and tolerance to higher salinity levels (Rothius *et al.*, 2013).

The present study aimed to evaluate Suez Canal state in relation to heavy metals pollution in water and fish collected. To consider the presence of heavy metals contaminants in musculature of samples of three different fish species: seabream (*S. aurata*), Seabass (*D. labrax*), and Meagre (*A. regius*) were randomly collected, from Suez Canal Farm to assess the seasonal physico-chemical characteristics of water resources as well as the hematological and biochemical status of the three different fish species to assess the pollution indices by heavy metals in water and their bioaccumulation in gills and muscles (the main edible tissue organs of fish).

MATERIALS AND METHODS

The present study was conducted at the Department of Animal Production and Fish Resources, Faculty of Agriculture, Suez Canal University and the practical part was applied at private fish farm at Suez Canal, Egypt. Water and fish samples of Seabream, Seabass, and Meagre were collected from Suez Canal

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area. The source of water samples was collected from Suez Canal and agricultural drainage water. Its ponds are earthen pond and each one about 1 acre. The private fish farm at Suez Canal was divided into 5 sectors, selected to represent a full coverage to the area under investigation:

Agricultural drainage water (sector A), mixed water (saline and agriculture drainage water) (sector B), gilthead seabream pond's water (sector C), seabass pond's water (sector D) and meagre pond's water (sector E).

Physical and chemical characteristics of water of fish farm's ponds and some heavy metals iron (Fe), copper (Cu), lead (Pb) and cadmium (Cd) in all sectors were measured simultaneously each month to be assembled latter in seasons.

Fish sampling:

Samples of three different fish species: Seabream (*S. aurata*), Seabass (*D. labrax*), and Meagre (*A. regius*), about 12 specimens totally which 4 specimens from each species, were randomly collected from three sectors (C, D and E). The biochemical and physiological changes of fish were measured monthly around the year from October 2018 to September 2019 and transacted seasonally later.

Water Physical Parameters:

Directly in the field, the water temperature (°C) and dissolved oxygen (DO) were immediately measured using a portable temperature probe and oxygen-meter, Omega (model DOH-SD1) instrument (U.S.A./Canada). Similarly, pH was measured in site also by using portable pH meter EXTECH (model, PH220A) instrument (U.S.A). However, Salinity (g/L) and Electric Conductivity (EC, mS/cm) as well as Total Dissolved Solids (TDS, mg/l) were measured instantly by using the portable Conductivity/TDS/Salinity Meter Lovibond SensoDirect (Con200) UK. All of these parameters were done in coincidence with the collection of water, and fish samples monthly around the year from October 2018 to September 2019, in order to be transacted seasonally later.

Blood parameters:

Detecting the changes in the blood parameters provide ability to predict the subsequent health of fish (Rehulka, 1996), since hematology becoming routine practice for determining the health status of the fish. Erythrocyte count (RBCs): Accurately 0.5 ml of the blood containing EDTA as an anticoagulant and using a double hemocytometer as described by Dacie and Lewis (1991) the erythrocyte count was done and expressed in million/ μ l.

Hematocrit value (Hct): It was determined by centrifuging the directly withdrawn heparinized blood, in the micro-hematocrit tube, at 5000 rpm for five minutes until the blood corpuscles were separated from the plasma (Britton, 1963).

Hemoglobin content (Hb): It was estimated by the colorimetric method using Boehringer Mannheim Kits as described by (Van kampen, 1961).

Erythrocyte indices: Erythrocyte status is also described by another derived three indices (Houston, 1990): Mean cell volume (MCV), it is the average volume of the red blood cells in μ^3 . It is calculated from hematocrit value (Hct) and red blood cell count.

$$MCV = Hct \times 10/RBCs (\times 10^6/\mu l) \dots \mu^3 \text{ or (fL)}$$

Mean cell hemoglobin (MCH), represents the average of hemoglobin content in each red blood cell. It is calculated from both hemoglobin content (Hb) and red blood cell count.

$$MCH = Hb \times 10/RBCs (\times 10^6/\mu l) \dots (\text{Pg})$$

Mean cell hemoglobin concentration (MCHC), represents the concentration of hemoglobin per unit volume of red blood cell. It is calculated from hemoglobin content (Hb) and hematocrit value (Hct).

$$MCHC = Hb \times 100/Hct (\text{g/dL})$$

Blood Biochemistry:

The blood which was collected without anticoagulants was left to be coagulated in clean and dry centrifuge tubes and then centrifuged for 15 minutes at 5000 rpm. The serum was separated, labeled and kept at -20°C in deep freezer for the following investigations:

Blood Glucose: Serum glucose concentration was measured by using Diamond diagnostics kit as described by Trinder (1969).

Blood Total Protein: The most widely used method of measuring serum protein is the biuret reaction. The intensity of the producing violet color is proportional to the concentration of protein. So, according to Henry (1974) the total serum protein of fish samples was determined by the colorimetric method using the *dp* international kits.

Blood Albumin Content: Albumin is generally measured by a dye-binding technique that utilizes the ability of albumin to form a stable complex with Bromocresol green dye (Busher, 1990a). The albumin content in serum of fish was determined by the colorimetric method using BCG (Bromocresol Green) Albumin Assay Kit Catalog Number MAK124.

Blood Globulin Content: The total globulin fraction is generally determined by subtracting the serum albumin protein content from the total serum protein content (Busher, 1990b): Serum globulin content = Serum total protein – Serum albumin

Blood Transaminases (AST and ALT): Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were determined calorimetrically according to the Reitman and Frankel (1957) method by using *dp* international kits.

Blood Urea: Blood urea was measured using the colorimetric assay using *dp* international kits according to Trinder (1969). The absorbance of color is proportional to the concentration of urea in samples.

Blood Creatinine: Serum creatinine level was measured using a colorimetric determination with deproteinization using *dp* international kits, according to Henry (1974).

Heavy Metals in Fish Muscles and gills: In the laboratory, dorsal muscles of ten fish specimens from

each fish-farm monthly take off. Fish muscles selected as they are the edible tissues for human consumption (Al-Ghanim *et al.*, 2016) and gills By using the hydrolysis step described in the NMKL-AOAC (Cunnif, 1995) official method and Flame Atomic Absorption Spectrometry (FAAS), heavy metals (Fe, Cu, Cd and Pb) were determined.

Statistical analyses:

Values were subjected to one-way ANOVA. All the statistical analyses were calculated using SPSS program version 20 (SPSS, Richmond, USA) as described by Dytham (1999). Values are given as Mean \pm SE. Mean was tested for significant differences at P-values \leq 0.05 (Duncan, 1955). To determine the effects of different seasons and sectors on heavy metals concentrations in water samples and fish tissues, statistical analysis was performed using the 2 x 5 factorial designs.

RESULTS

In the present study, a comparative investigation was given to elucidate the impact of some heavy metals in water quality, physiological status, biochemical parameters and accumulation of heavy metals in flesh and gills of the following reared fish species gilthead seabream (*S. aurata*), seabass (*D. labrax*) fed

commercial diet (40% protein), and meagre (*A. regrius*). Samples were collected from private fish farm at Suez Canal, Ismailia, Egypt. The water sources of this farm were about 80% from Suez Canal and 20% from agricultural drainage water. The farm is divided into 1 acre separated earthen ponds and each 32 ponds have branched irrigation canal from the main irrigation canal and branched to drainage canal which drain into Suez Canal after proper sterilization transactions for the water. Firstly, the physical characteristic features of the water were measured. The monthly collecting data around the year from October 2018 to September 2019 were showed in the followings.

Physical Features of water: Water temperature (sector A, B, C, D and E) ranged between 12 – 31°C in the Suez Canal region from Oct. 2018 to Sep. 2019 (Table 1). Water dissolved oxygen content in the investigated water samples was recorded in Sep. for sector A, B, C, D, and E was between 3.25 - 4.75. Salinity measurements were 4.55-8.00, 10.00-18.59, 36.00-40.00, 37.00-41.00 and 22.00-30.50 ppt for sectors A, B, C, D, and E, respectively. In the present work it was found that, the pH measuring of each sector's water indicated its slight Alkalinity with range values between 6.50 to 7.95 (Table 1).

Table (1): The range of water temperature (°C), dissolved Oxygen (mg/l) salinity, and pH of water samples collected monthly from sectors A, B, C, D and E from a private fish farm in the Suez Canal region from Oct. 2018 to Sep. 2019

Month	Sector A	Sector B	Sector C	Sector D	Sector E
Temp. (°C)	12.50- 30.75	12 - 31	13.00 - 30.75	13.50 - 30.75	13.50 - 29.25
DO (mg/l)	3.85 - 4.75	3.25 - 4.75	3.75 - 4.50	3.80 - 4.50	3.40 - 4.75
Salinity (ppt)	4.55 - 8.00	10.00 - 18.59	36.00 - 40.00	37.00 - 41.00	22.00 - 30.50
pH	6.50 - 7.40	7.20 - 7.95	7.25 - 7.75	7.13 - 7.60	7.15 - 7.75

Blood parameters of the investigated fish:

The erythrocyte count (RBCs): On the current study it was found that the highest value of the erythrocyte count (RBCs) for *S. aurata* was $2.65 \times 10^6/\mu\text{l}$ on Jun., while the lowest value was $0.57 \times 10^6/\mu\text{l}$ on Mar. in sector (C), and the highest value for *D. labrax* was found to be $2.80 \times 10^6/\mu\text{l}$ on Dec., while the lowest value

recorded was $1.39 \times 10^6/\mu\text{l}$ on Mar. in sector (D), and for *A. regrius* the highest value was found to be $2.26 \times 10^6/\mu\text{l}$ on Jun. and the lowest value recorded was $0.79 \times 10^6/\mu\text{l}$ on Jan. in sector (E) (Figure 1). RBCs count of different fish species were significantly differed ($P < 0.05$) in-between each investigated fish.

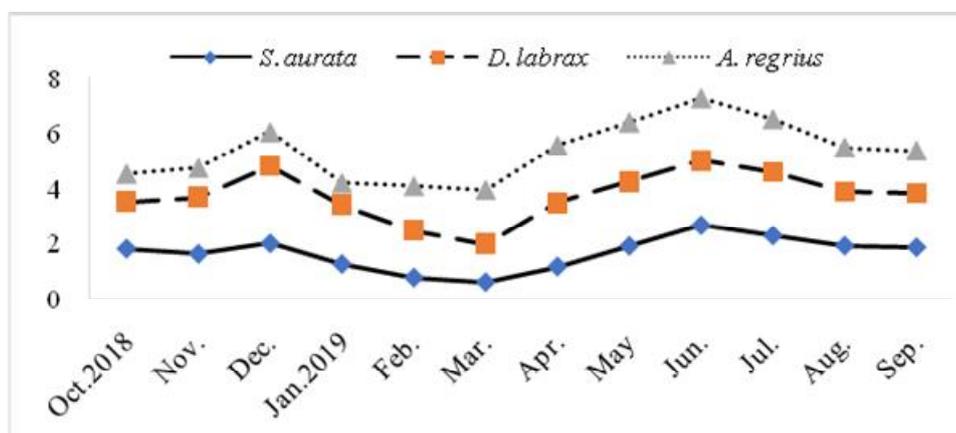


Figure (1): Erythrocyte count ($\times 10^6/\mu\text{l}$) of different fish species, *S. aurata*, *D. labrax* and *A. regrius* collected monthly from private fish farm in the Suez Canal region from Oct. 2018 to Sep. 2019

Blood hemoglobin content (Hb): At the current investigation the highest value of blood hemoglobin content (Hb) for *S. aurata* was high to be 11.87 g/dL on Aug, while the lowest value recorded was 2.30 g/dL on May in sector (C), the highest for *D. labrax* was found to be 7.52 g/dL on Aug., and the lowest value recorded

was 3.762 g/dL on Sep in sector (D), and for *A. regrius* the highest value was found to be 10.27 g/dL on Aug, while the lowest value recorded was 1.611 g/dL on Dec in sector (E) (Figure 2). Blood hemoglobin content significantly differed in-between each investigated fish ($p < 0.05$).

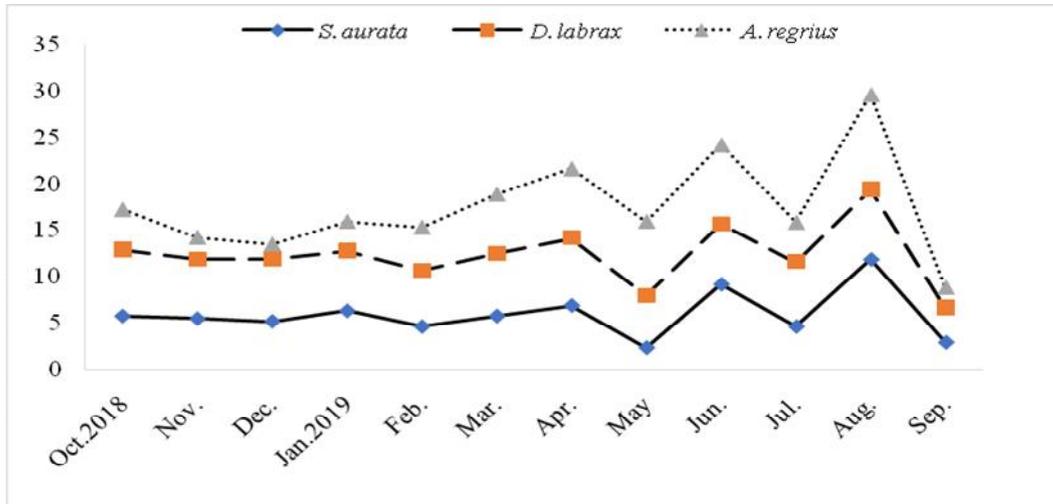


Figure (2): Hemoglobin content (g/dL) of different fish species, *S. aurata*, *D. labrax* and *A. regrius* collected monthly from private fish farm in the Suez Canal region from Oct. 2018 to Sep. 2019

Blood hematocrit (Hct): At the present study the highest value recorded of blood hematocrit (Hct) in the blood for *S. aurata* was 43.16% on Aug., while the lowest value was 12.85% on Mar. in sector (C), the highest value for *D. labrax* was found to be 47.97% on Dec. and the lowest value recorded was 28.32% on Mar.

in sector (D), the highest value for *A. regrius* was found to be 69.83% on Apr., and the lowest value recorded was 27.44% on Dec. in sector (E) (Figure 3). With a significant difference between each investigated fish ($P < 0.05$).

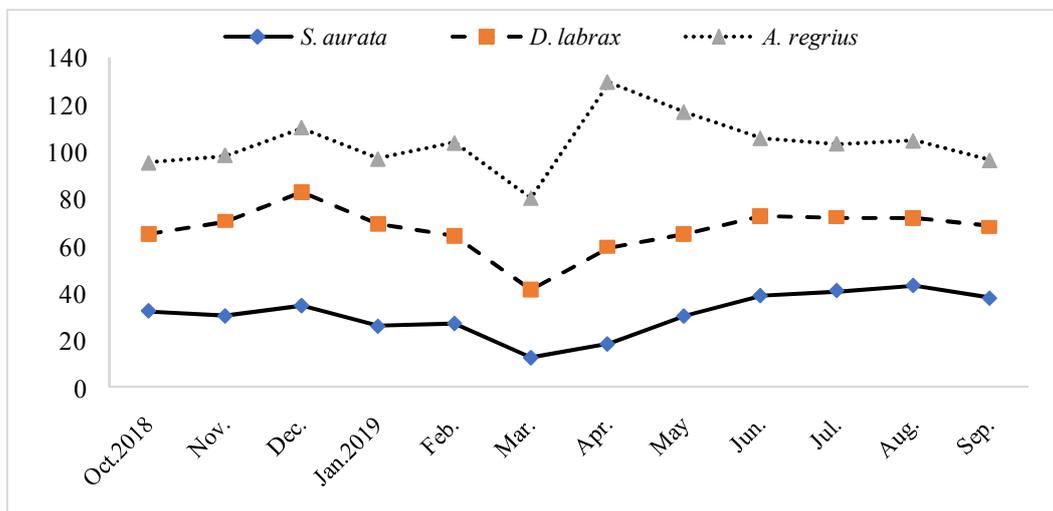


Figure (3): Hematocrit value (%) of different fish species, *S. aurata*, *D. labrax* and *A. regrius* collected monthly from private fish farm in the Suez Canal region from Oct. 2018 to Sep. 2019

Red blood cell indices:

Mean cell volume (MCV): At the present investigation the highest value of Mean cell volume (MCV) noticed in the blood for *S. aurata* was 373.959 μ^3 on Feb., while the lowest value was 146.569 μ^3 on Jun in sector (C), the highest value for *D. labrax* was found to be 229.312

μ^3 on Feb., and the lowest value recorded was 135.023 μ^3 on Jul. in sector (D), the highest value of MCV for *A. regrius* was found to be 348.85 μ^3 on Apr. and the lowest value recorded was 65.60 μ^3 on Oct. in sector (E) (Figure 4). With a significant difference between each investigated fish ($P < 0.05$).

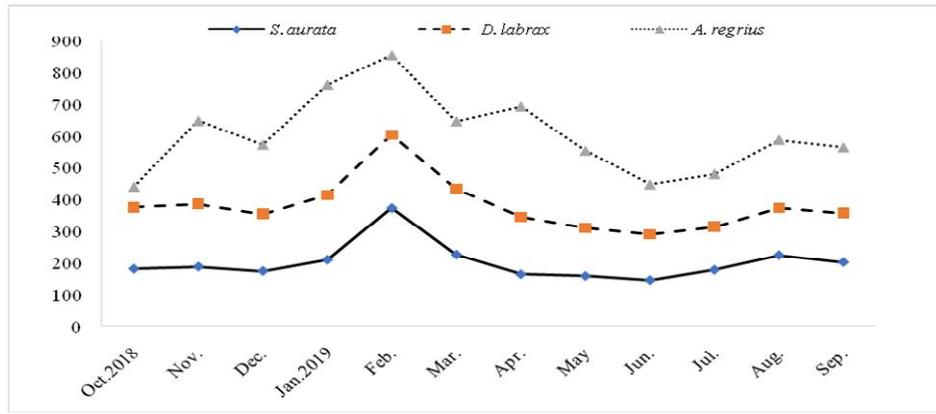


Figure (4): Mean cell volume (MCV, μ3): of different fish species, *S. aurata*, *D. labrax* and *A. regrius* collected monthly from private fish farm in the Suez Canal region from Oct. 2018 to Sep. 2019

Mean cell hemoglobin (MCH): During the progressing studies it was found that the highest value of Mean cell hemoglobin (MCH) in the blood for *S. aurata* was 101.53 Pg on Mar., while the lowest value was 12.177 Pg on May in sector (C), the highest value for *D. labrax* was found to be 101.125 Pg on Oct., and the lowest

value recorded was 19.234 Pg on Sep. in sector (D), while the highest value for *A. regrius* was found to be 64.15 Pg on Aug. and the lowest value recorded was 9.56 Pg on Oct. in sector (E) (Figure 5). With a significant difference between each investigated fish ($P < 0.05$).

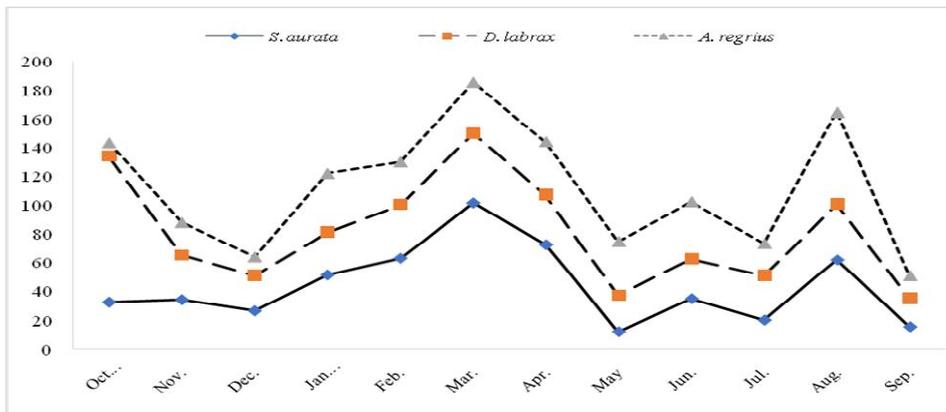


Figure (5): Mean cell hemoglobin (MCH, Pg) of different fish species, *S. aurata*, *D. labrax* and *A. regrius* collected monthly from private fish farm in the Suez Canal region from Oct. 2018 to Sep. 2019

Mean cell hemoglobin concentration (MCHC): In the present investigation it was found that the highest value of Mean cell hemoglobin concentration (MCHC) in the blood for *S. aurata* was 46.19 on Mar., while the lowest value was 7.626 on May in sector (C), the highest value for *D. labrax* was found to be 52.647 on Oct. and the

lowest value recorded was 12.491 on Sep. in sector (D), while the highest value for *A. regrius* was found to be 31.73 on Aug. and the lowest value recorded was 6.44 on Dec. in sector (E) (Figure 6). With a significant difference between each investigated fish ($P < 0.05$).

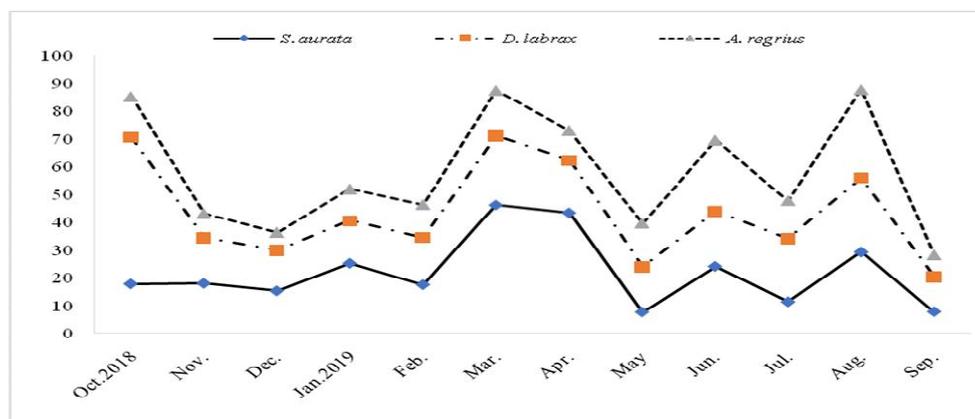


Figure (6): Mean cell hemoglobin concentration (MCHC, g/dL) of different fish species, *S. aurata*, *D. labrax* and *A. regrius* collected monthly from private fish farm in the Suez Canal region from Oct. 2018 to Sep. 2019

Blood biochemistry

The blood consists of blood cells, erythrocytes and leukocytes in addition of blood platelets all are suspended with other organic molecules; proteins, sugars, urea, creatinine, enzymes as well as ions all are dissolved in a liquid medium, blood plasma.

Total blood protein: In the current studies the highest value recorded of total blood protein for *S. aurata* was 4.129 g/dL on Dec, while the lowest value was 1.586

g/dL on Apr. in sector (C), while the highest value for *D. labrax* was found to be 4.01 g/dL on Dec. and the lowest value recorded was 3.12 g/dL on Feb. in sector (D), while the highest value for *A. regrius* was found to be 5.195 g/dL on Mar, and the lowest value recorded was 2.146 g/dL on Sep. in sector (E) (Figure 7). With a significant difference between each investigated fish ($P < 0.05$).

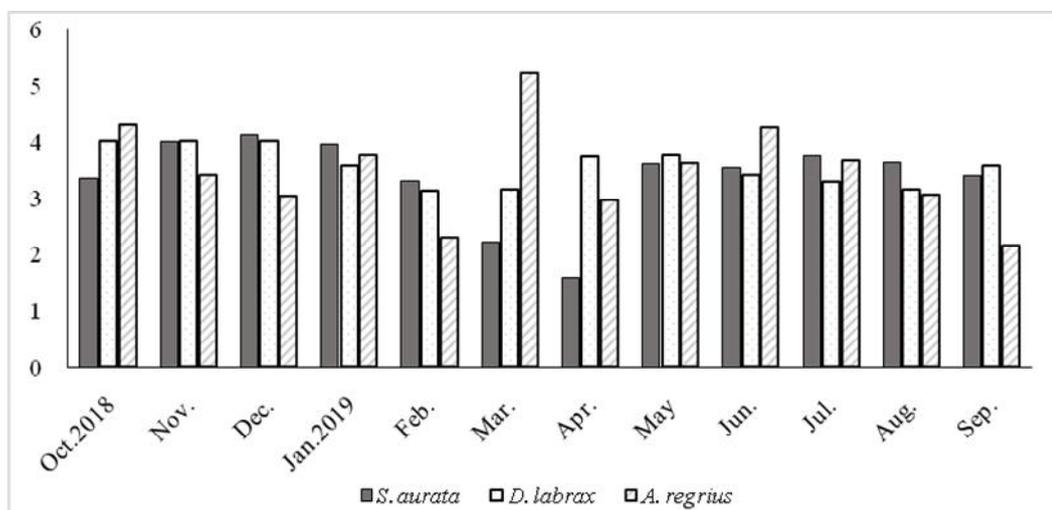


Figure (7): Total blood protein (g/dL) of different fish species, *S. aurata*, *D. labrax* and *A. regrius* collected monthly from private fish farm in the Suez Canal region from Oct. 2018 to Sep. 2019

Globulin: In the present investigation it was found that the highest value of albumin for *S. aurata* was 1.16 g/dL on Aug., while the lowest value was 0.28 g/dL on Dec. in sector (C), while the highest value for *D. labrax* was found to be 1.067 g/dL on Oct. and the lowest value

recorded was 0.40 g/dL on Jun. in sector (D). The highest value for *A. regrius* was found to be 1.69 g/dL on Aug. and the lowest value recorded was 1.05 g/dL on Nov. in sector (E) (Figure 8). With a significant difference between each investigated fish ($P < 0.05$).

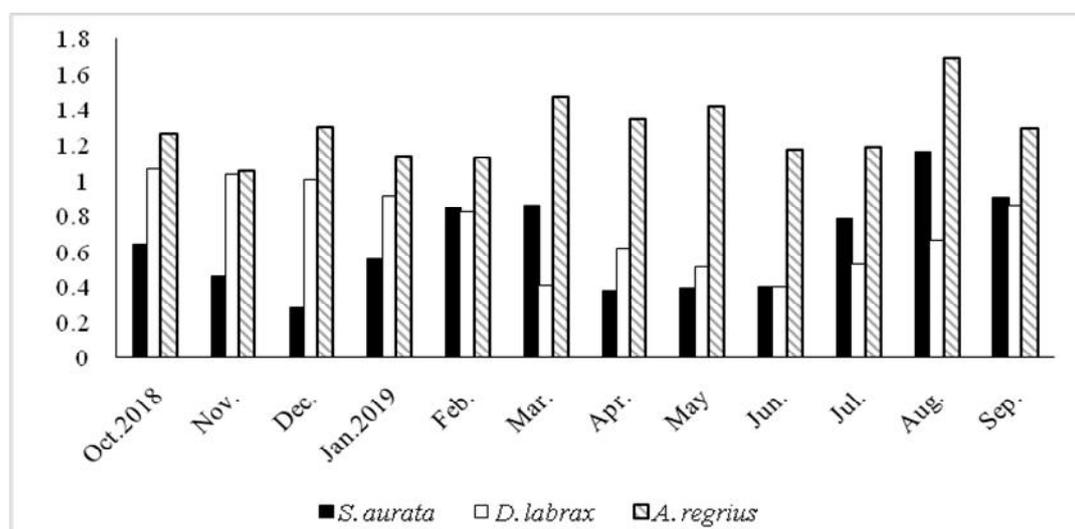


Figure (8): Globulin serum contents (g/dL): of different fish species, *S. aurata*, *D. labrax* and *A. regrius* collected monthly from private fish farm in the Suez Canal region from Oct. 2018 to Sep. 2019

Albumin: During the present investigation it was found that the highest value of globulin for *S. aurata* was 4.10 g/dl on Jan., while the lowest value was 1.69 g/dl on Mar. in sector (C), while the highest value for *D. labrax* was found to be 3.88 g/dl on Apr. and the lowest value

recorded was 2.06 g/dl on Mar. in sector (D). The highest value for *A. regrius* was found to be 3.715 g/dl on Mar. and the lowest value recorded was 1.512 g/dl on Sep. in sector (E) (Figure 9). With a significant difference between each investigated fish ($P < 0.05$).

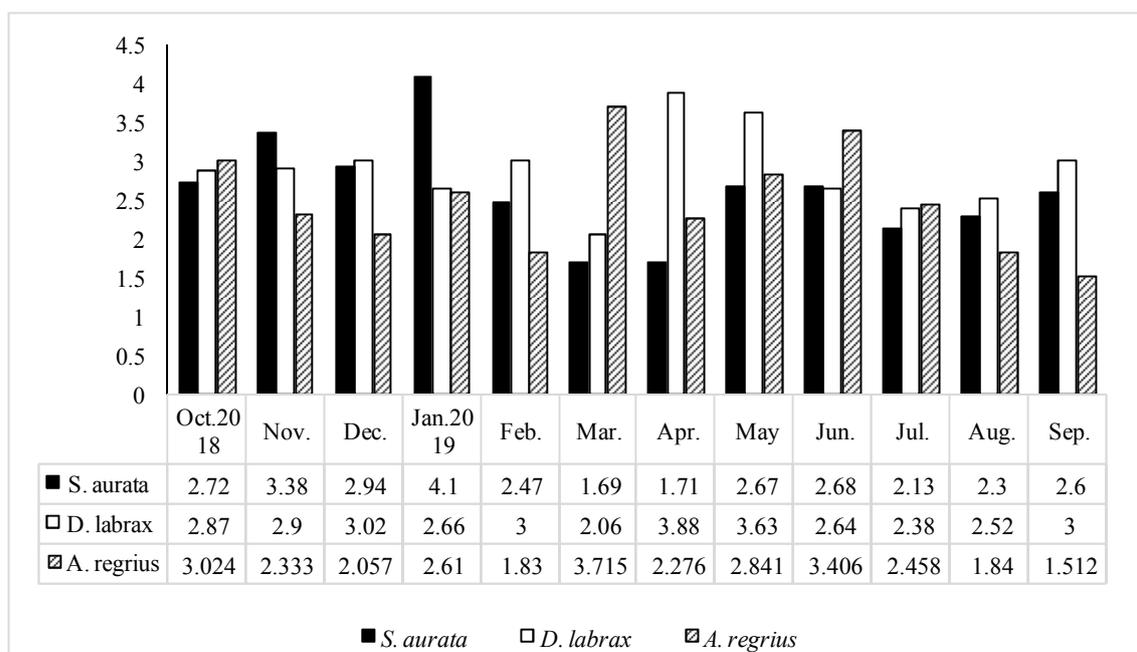


Figure (9): Albumin serum content (g/dl) of different fish species, *S. aurata*, *D. labrax* and *A. regrius* collected monthly from private fish farm in the Suez Canal region from Oct. 2018 to Sep. 2019

Blood glucose content: At the present study the highest value recorded in the glucose in the blood for *S. aurata* was 235.71 mg/dl on Feb., while the lowest value was 41.48 mg/dl on Aug. in sector (C). Also, the highest value for *D. labrax* was found to be 198.89 mg/dl on Oct., and the lowest value recorded was 76.89 mg/dl on

Aug. in sector (D). The highest value for *A. regrius* was found to be 125.96 mg/dl on Jun. and the lowest value recorded was 26.7 mg/dl on Sep. in sector (E), (Figure 10). With a significant difference between each investigated fish ($P < 0.05$).

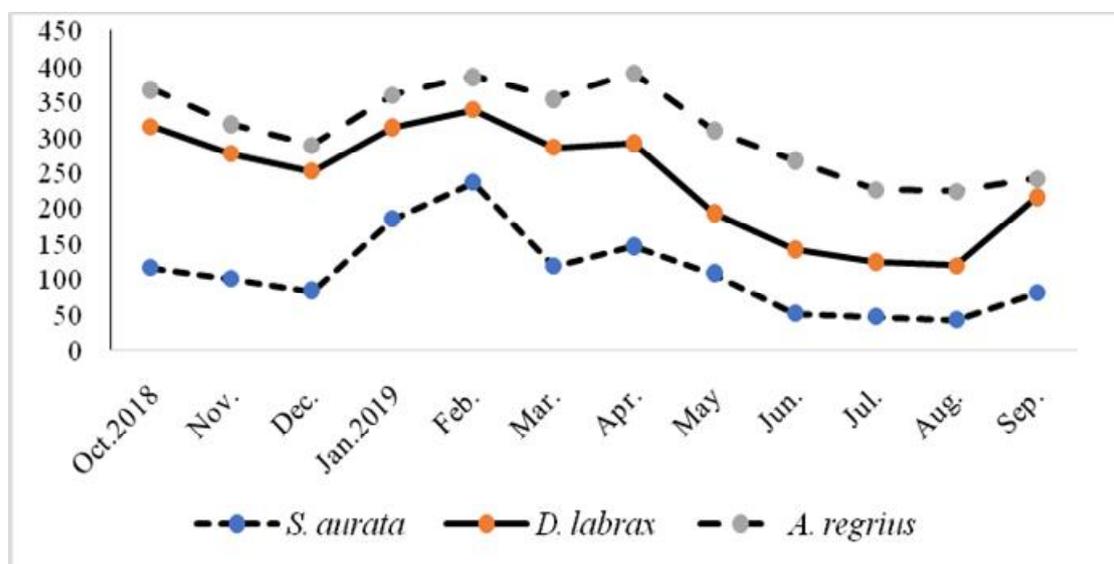


Figure (10): Serum glucose contents (mg/dl) of different fish species, *S. aurata*, *D. labrax* and *A. regrius* collected monthly from private fish farm in the Suez Canal region from Oct. 2018 to Sep. 2019

Blood Urea Nitrogen (BUN): At the present investigation the highest value of blood urea nitrogen (BUN) noticed for *S. aurata* was 8.80 mg/dl on Aug., while the lowest value was 4.16 mg/dl on Apr. in sector (C), while the highest value for *D. labrax* was found to be 3.31mg/dl on Aug. The lowest value recorded was

1.67 mg/dl on Dec. in sector (D), while the highest value for *A. regrius* was found to be 12.95 mg/dl on Mar., and the lowest value recorded was 2.882 mg/dl on Aug. in sector (E) (Figure 11). With a significant difference between each investigated fish ($P < 0.05$).

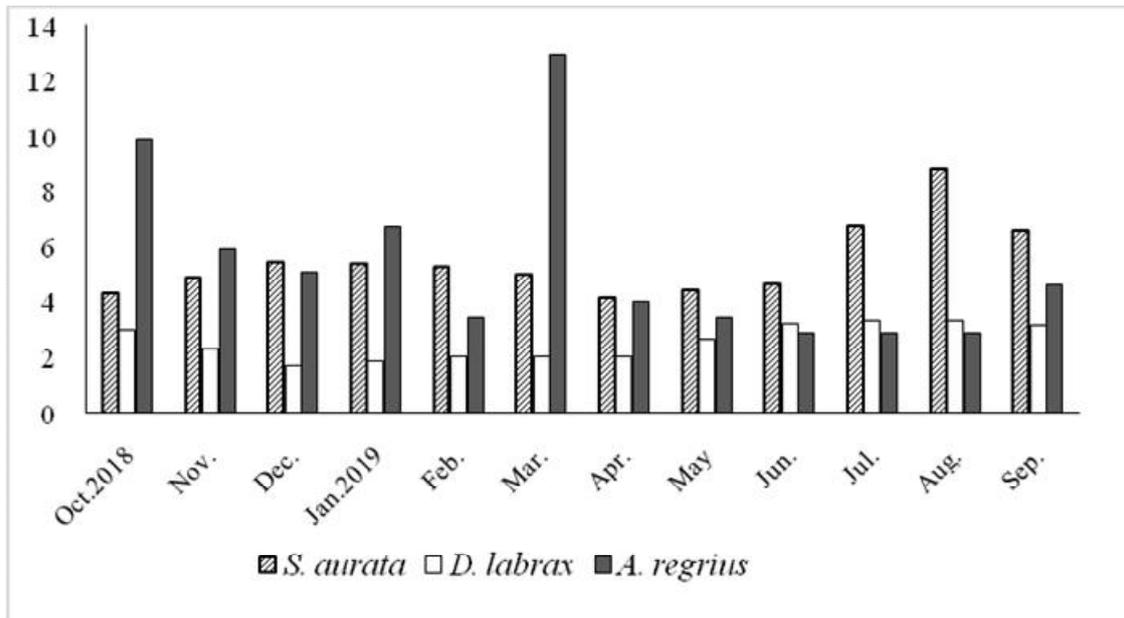


Figure (11): Serum Urea contents (mg/dl): of different fish species, *S. aurata*, *D. labrax* and *A. regrius* collected monthly from private fish farm in the Suez Canal region from Oct. 2018 to Sep. 2019

Blood Creatinine: During the progressing studies it was found that the highest value of blood creatinine for *S. aurata* was 0.001 mg/dl on Dec. and the lowest value was 0.0002 mg/dl on Oct. in sector (C). The highest value for *D. labrax* was found to be 0.0019 mg/dl on Dec. and the lowest value recorded was 0.0012 mg/dl

on Mar. in sector (D), while the highest value for *A. regrius* was found to be 0.0010 mg/dl on Aug. and the lowest value recorded was 0.0003 mg/dl on Sep. in sector (E), (Figure 12) with a significant difference between each investigated fish ($P < 0.05$).

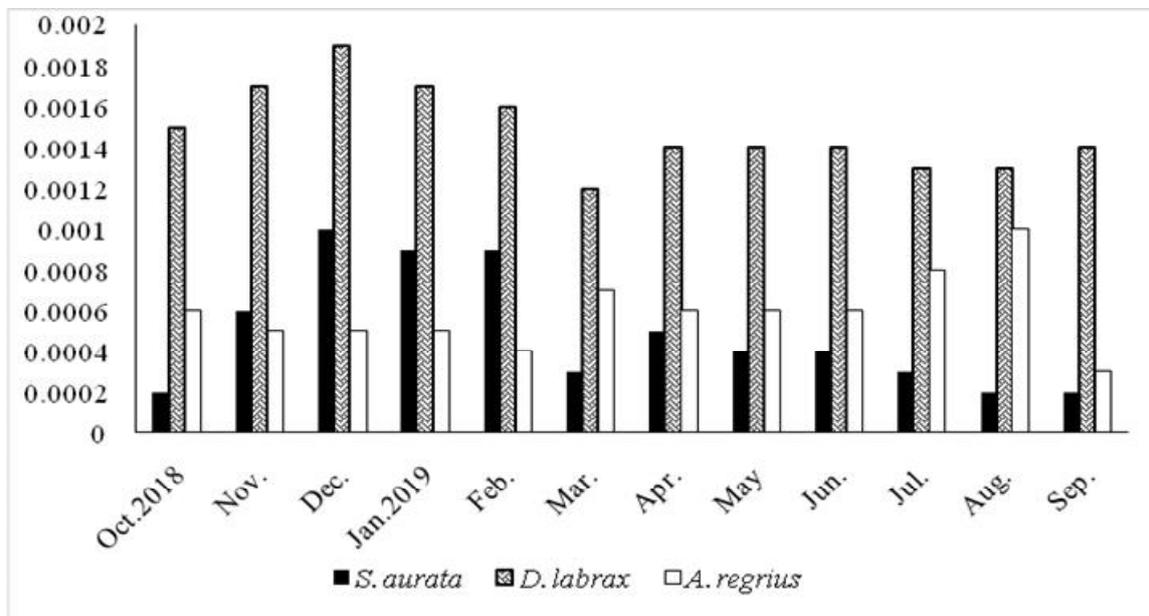


Figure (12): Serum creatinine contents (mg/dl): of different fish species, *S. aurata*, *D. labrax* and *A. regrius* collected monthly from private fish farm in the Suez Canal region from Oct. 2018 to Sep. 2019

Alanine transaminase (ALT) and Aspartate transaminase (AST): During the present investigation it was found that the highest value of alanine amino transaminase (ALT) for *S. aurata* was 139.67 U/l on Dec., while the lowest value was 35.54 U/l on Aug. in sector (C), while the highest value for *D. labrax* was

found to be 45.75 U/l on Jun. and the lowest value recorded was 34.47 U/l on Mar. in sector (D), while the highest value for *A. regrius* was found to be 42.371 U/l on Feb. and the lowest value recorded was 20.951 U/l on Sep. in sector (E), (Figure 13). With a significant difference between each investigated fish ($P < 0.05$).

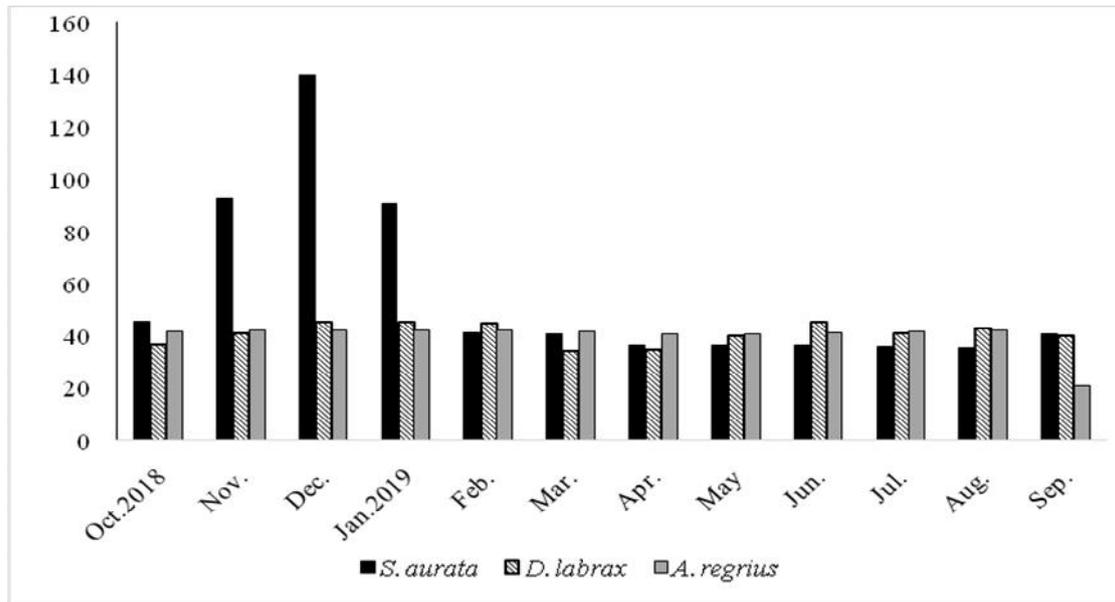


Figure (13): ALT (U/l) of different fish species, *S. aurata*, *D. labrax* and *A. regrius* collected monthly from private fish farm in the Suez Canal region from Oct. 2018 to Sep. 2019

During the progressing studies it was found that the highest value of aspartate amino transaminase (AST): for *S. aurata* was 114.91 on Dec., while the lowest value was 51.56 U/l on Apr. in sector (C), while the highest value for *D. labrax* was found to be 105.22 U/l on Apr. and the lowest value recorded was 75.52 U/l

on Feb. in sector (D), while the highest value for *A. regrius* was found to be 92.66 U/l on Mar. and the lowest value recorded was 45.729 U/l on Sep. in sector (E) (Figure 14). With a significant difference between each investigated fish ($P < 0.05$).

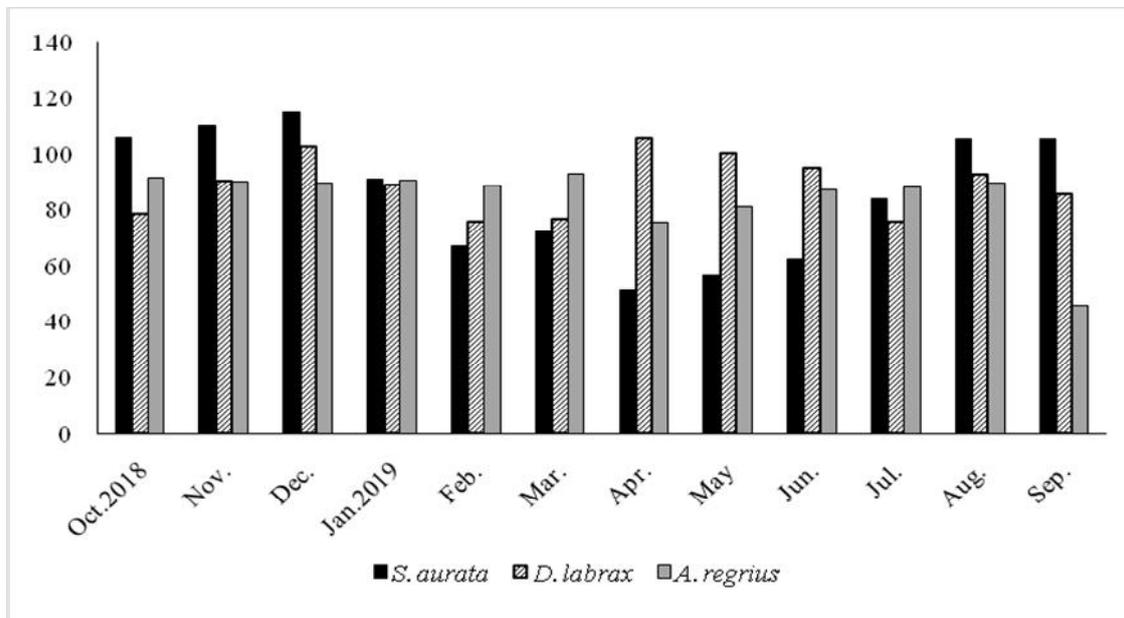


Figure (14): AST (U/l) of different fish species, *S. aurata*, *D. labrax* and *A. regrius* collected monthly from private fish farm in the Suez Canal region from Oct. 2018 to Sep. 2019

Heavy metals level in water: In the current work the heavy metal load of iron (Fe), copper (Cu), cadmium (Cd) and lead (Pb) in aquaculture water of the investigated fish farm sectors is given in tables (26). It was found that, the highest contents of Fe (0.540mg/L) was recorded in sector (B), Cu and Pb (1.44 and 0.032mg/L) were measured in sector (D), and Cd (0.168 mg/L) was in sector (c). Otherwise, the lowest content

of Fe (0.312 mg/l) was noticed in sector (C), Cu (0.977 mg/L) was recorded in sector (E), Cd (0.001 mg/L) in sector (B, D, and E) respectively and Pb (0.008 and 0.008 mg/L) was measured in sector (A and E). It must be pointed out that, the four heavy metals load variances in-between the fish farm sectors of significant ($P < 0.005$) (Table 2).

Table (2): Heavy metals concentrations (ppm) in water samples from different seasons (winter and summer) and different sectors in Suez Canal region

		Fe (ppm)	Cu (ppm)	Cd (ppm)	Pb (ppm)
Interaction effect of seasons & sectors					
Winter	Sector A	0.580 ^a ± 0.138	1.173 ^{abc} ± 0.068	0.004 ± 0.002	ND
	Sector B	0.5103 ^a ± 0.066	1.217 ^{abc} ± 0.0609	0.001 ± 0.0003	0.017 ^{bc} ± 0.006
	Sector C	0.441 ^a ± 0.057	1.098 ^{bc} ± 0.086	0.001 ± 0.0003	0.008 ^{cd} ± 0.004
	Sector D	0.410 ^a ± 0.043	1.305 ^{ab} ± 0.034	ND	0.03 ^{ab} ± 0.004
	Sector E	0.446 ^a ± 0.038	1.1503 ^{bc} ± 0.029	0.001 ± 0.0003	0.006 ^{cd} ± 0.003
Summer	Sector A	0.423 ^a ± 0.067	1.636 ^a ± 0.063	0.003 ± 0.0003	0.016 ^{bc} ± 0.008
	Sector B	0.573 ^a ± 0.035	1.23 ^{abc} ± 0.051	0.002 ± 0.0002	0.03 ^{ab} ± 0.006
	Sector C	0.183 ^b ± 0.097	1.153 ^{bc} ± 0.084	0.335 ± 0.333	0.022 ^{abc} ± 0.001
	Sector D	0.465 ^a ± 0.038	1.575 ^{ab} ± 0.0809	0.003 ± 0.0015	0.035 ^a ± 0.0009
	Sector E	0.506 ^a ± 0.023	0.804 ^c ± 0.405	0.001 ± 0.0003	0.011 ^{cd} ± 0.006
Permissible limits		≤ 30 [5]	2 [6]	0.003 [6]	0.01 [6]

Mean values of the same column within each part of the table with different superscript are significantly different

Heavy metals accumulation in gills and muscles:

According to the present results, there were insignificant differences between the detected heavy metals (Fe, Cu, Cd, and Pb) levels in the gills of fish species cultivated in the three investigated fish farm sectors (C, D, and E). The average highest content of Fe, and Cd (9.551 and 0.014 mg/g), were recorded in seabass, but for Cu

(0.395 mg/ g) was noticed in gilthead seabream and pb (0.196 mg/g) were measured in meagre. On the other hand, the average lowest values of Fe and Cd gill contents (4.751, and 0.0004 mg/g) were done in gilthead seabream but for Cu and Pb (0.219 and 0.005 mg/g) were found in seabass (Table 3).

Table (3): Heavy metals concentrations (ppm) in gills and muscles of fish collected from private fish farm in the Suez Canal region

	<i>S. aurata</i>	<i>D. labrax</i>	<i>A. regius</i>	The permissible limits in fish tissue (ppm)
Fe gills	4.751 ^b ± .499	9.551 ^a ± .428	8.673 ^a ± .489	≤ 30 (FAO,1992)
Fe muscles	3.803 ^b ± .449	6.630 ^a ± .204	2.889 ^b ± .083	≤ 30 (FAO,1992)
Cu gills	0.395 ± .0891	0.219 ± .023	0.265 ± .019	1 (WHO, 2003)
Cu muscles	0.083 ^b ± .009	0.160 ^a ± .019	0.107 ^b ± .014	1 (WHO, 2003)
Pb gills	0.009 ^b ± .003	0.012 ^b ± .004	0.196 ^a ± .157	0.05 (WHO, 2003)
Pb muscles	0.016 ± .007	0.005 ± .003	ND	0.05 (WHO, 2003)
Cd gills	0.0004 ^b ± .000	0.014 ^a ± .005	0.002 ^b ± .001	0.003 (WHO, 2003)

Mean values of the same row with different superscript are significantly different (p≤0.05)

There were significant differences (P≤0.05) between the detected heavy metals (Fe, Cu, and Pb) levels in the dorsal muscles of fish species cultivated in the three investigated fish farm sectors (C, D, and E). The average highest content of Fe, and Cu (6.630 and 0.160 mg/g) were recorded in seabass, but for pb (0.016 mg/g) were measured in gilthead seabream. On the other hand, the average lowest values of Fe muscle contents (2.889 mg/g) were done in meagre but for Cu and Pb (0.083 and 0.012 mg/g) were found in seabream and seabass, respectively (Table. 3).

DISCUSSION

The present study assessed to evaluate Suez Canal state in relation to heavy metals pollution in water and fish collected. To consider the fish physiology, biochemistry and heavy metals contaminants in musculature of samples of three different fish species: seabream (*S. aurata*), Seabass (*D. labrax*), and Meagre (*A. regius*). As fish is a coldblooded animal, its body temperature changes according to that of environment affecting its metabolism and physiology and ultimately affecting their production. Higher temperature increases

the rate of bio-chemical activity in fish and so increases their oxygen demand. It further decreased solubility of oxygen and also increased level of ammonia in water. However, pond's water temperature was fluctuated significantly with different seasons between 12.00 °C at Feb. and 31 °C at Aug. in all sectors (A, B, C, D and E). Since, water temperatures in ponds are related to solar radiation and air temperatures so, water temperatures in ponds generally are quite predictable by season and location (Boyd, 1990). Generally, the decrease in oxygen concentrations in water bodies may be due to oxygen consumption by organisms, decomposition organic matter and oxidation of chemical effluents discharged from different factories around this area as previously reported by Aboul Ela *et al.* (1990).

In the current work, the average level of dissolved oxygen was ranged between 3.25 to 4.75 mg/l in fish sectors (A, B, C, D and E). However, caution should be considered, since dissolved oxygen concentration is never constant, it can be depleted when water contains decayed organic matters and/or plant population becomes too dense (PHILMINAQ, 2004). Toxic effects as a consequence of increasing salinity in dry seasons than wet season cause physiological changes, resulting in a loss (or gain) of species. Indirect changes can occur where increasing salinity modifies community structure and function by removing (or adding) taxa that provide refuge, food or modify predation pressure. Other factors such as water-logging or loss of habitat may interact with salinity or have a more immediate impact on species richness (Clunie *et al.* 2002). The pH values ranged from 6.50 to 7.95, in the four sectors A, B, C, D and E which represents agricultural drainage water (sector A), mixed water (sector B), *S. aurata* water pond (sector C), *D. labrax* water pond (sector D) and *A. regrius* water pond (sector E). El-Wakeel and Wahby, (1970) found that the increase of pH values in water in winter than in summer is related to increase in primary production by increase in photosynthesis involving the uptake of free carbon dioxide from carbonate-bicarbonate buffer system and precipitation of calcium carbonate. On the other hand, the lower values of pH in summer are associated with decomposition of organic matter (Elewa and Mahdi, 1988).

Many researchers reported that there are variations in blood parameters values that can be attributed to many factors such as age, size of fish, nutrition state, season, spawning, sex, and genetic variation. The high value of RBCs recorded in June and July (2.65 and $2.27 \times 10^6/\mu\text{l}$) for seabream, from April to July for seabass and meagre, that agreed with Poston (1966) who mentioned that the number of red blood cells and hemoglobin concentration tends to increase with length and age. Jawad *et al.* (2004) found that increased values of RBC, Hct and Hb in relation to increase of fish size. Moreover, in fish, the hematological parameters are closely linked with the metabolic level connected with the size (Fazio *et al.*, 2015). Cyriac *et al.* (1989) found that fish severely exposed to copper shown a prevalent in hematocrit and hemoglobin form in blood, may be due to changes in

blood parameters which result in erythrocyte swelling or by release of great red blood cells from the spleen. Our study agrees with Scott and Rogers (1981) who found that the increased Hct values are associated with a rise in the number of cells per unit blood volume. Elevated values of Hct have been attributed to the redistribution of water from the general circulation to tissues and spleen concentration. Hct recorded high value in autumn and winter for seabream and seabass (spawning season). This increase has been interpreted in relation to the high-energy requirements of fish during the breeding season (Jawad *et al.*, 2004). The present study agrees with Swift (1981) who found that the decrease in blood parameters is accompanied by an increase in MCV and MCH and with insignificant change in MCHC which may be due to the hemolytic action which led to fluid loss to the tissues with subsequent decrease in the plasma volume. High value of Hb recorded in August for all species related to a high respiratory demand due to high temperature that was agreement with De Pedro *et al.* (2005) who showed the Hb concentration increase in blood to carry more oxygen in high temperature. High value recorded of MCH and MCHC in summer may be related to hemoglobin value. MCV recorded high value in summer and winter may be related to Hct values. Changes in observed values of these parameters may reflect the responses of fish to changes in their environment.

Measurement of biochemical and physiological parameters is a commonly used diagnostic tool in aquatic toxicology and biomonitoring (Xiaoyun *et al.*, 2009). The ranges of normal values of the key biochemical parameters are still undefined for different species in different aquaculture conditions (Barcellos *et al.*, 2003). The physiological and biochemical characteristics of fish blood are easily modified by environmental changes (Atamanalp *et al.*, 2002). Moreover, the sampling technique, analysis methods, fish age, habitat, and diet can also affect blood parameters (Sakomoto *et al.*, 2001). The ranges of serum biochemistry varied from species to species and can be influenced by many biotic and abiotic factors such as water temperature, seasonal pattern, food, age, and sex of the fish (Jawad *et al.*, 2004). In the present investigation it was found that, the total serum protein of sea bream reared in the marine fish farms was fluctuated between (4.129 and 1.586 g/dL) as the highest value at Dec. and the lowest at Apr. Insignificant differences were observed in total protein for sea bass. The present results were also in accordance with those given by Patriche *et al.* (2009), concerning variation of the total blood protein with water temperature in different seasons, highest serum protein level was recorded in summer (high-water temperature). The increased plasma protein concentration can be caused by structural liver alternations that reduce aminotransferase activity, with concurrent reduction in deamination capacity (Kavadias *et al.*, 2004). Hrubec *et al.* (2001) stated that protein level in striped bass increased with age. In the present studies, blood glucose levels were greatly varied, it was greatly fluctuated between 41.48, 85.58 and 26.7 mg/dl as a minimum

value, in Aug. May and Sept. while, 235.71, 198.89 and 125.96 mg/dl as a maximum, in Feb, Oct and June for seabream, seabass and meagre respectively. Increased blood glucose and protein level was recorded in *A. subrostratus*; this may be probably due to an increased depletion of liver glycogen (Ojolic *et al.*, 1995). Our findings confirm those of Coz-Rakovac *et al.* (2005) who reported a significantly elevated glucose level in farmed *D. labrax*. Blood glucose concentration decrease in fish is based on the age and size of fish (Hrubec *et al.*, 2001). In the present studies it was found that, blood urea content significantly varied in-between the investigated fish. Its lowest average level (4.16, 1.67 and 2.88 mg/dl) was noticed during Apr., Dec. and Jun. to Aug. while, the highest urea level (8.80 Aug. 3.31 Aug. and 12.95 Mar.) were noticed almost in seabream sea bass and meagre respectively. It could be attributed to disturbance in the fish kidney function (Lockhart and Metner, 1984) and/or to gill dysfunction (Murray *et al.*, 1990). Creatinine recorded insignificant differences between different months for all species showed slight increase in winter due to spawning season of seabream and seabass. Also, that may be related to high demand of energy needed for spawning so brood stock used all energy storage and amino acid to produce more energy for spawning process that agreed with Srivastava *et al.* (1999) who reported that the significantly higher activities of c-AST and m-AST during the spawning phase together with the high activities of alanine aminotransferase (ALT) of the same fish species (Srivastava, 1997). It is therefore possible that the flow of metabolites through AST and ALT is enhanced by utilization of more amino acids through the malate-aspartate shuttle during the spawning phase. Significant differences between different months for all species were observed for ALT and AST values, which were the highest in winter months for all for ALT while, AST was the lowest value in Sep. for meagre fish. Several studies have reported on the adaptive changes in metabolic enzymes as a function of temperature acclimation in fish (Lin and Somero, 1995). Particularly in fish, ALT has been found to show significant alterations under the influence of environmental factors.

The pollution of the aquatic environment with heavy metals has become a worldwide problem during recent years, because they are indestructible and most of them have toxic effects on organisms (MacFarlane and Burchett, 2000). Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate in aquatic ecosystems (Censi *et al.*, 2006). Pollution of the aquatic environment by inorganic chemicals has been considered a major threat to the aquatic organisms including fish. The agricultural drainage water containing pesticides and fertilizers and effluents of industrial activities and run offs in addition to sewage effluents supply the water bodies and sediment with huge quantities of inorganic anions and heavy metals (ECDG, 2002).

Insignificant differences between winter and summer seasons were observed for heavy metals concentrations (ppm) in water samples from different

sites in Suez Canal, except lead (Pb). Differences between the different sectors were observed for heavy metals concentrations (ppm) in water samples except for cadmium (Cd) content between sectors. The combined effect of seasons and different sectors showed significant differences between seasons and the different sectors (A, B, C, D, and E) except for cadmium (Cd) content. The lowest values of Fe were recorded during summer in sector C. The highest values of Cu were recorded during summer in sector A, insignificant differences were observed for Cd between seasons and sectors. Finally, the highest values of Pb were recorded during summer in sector D. Values (Cu, Cd and Pb) were nearly in the Permissible limits of WHO (2011) and Fe according to Meade (1989). Similar increase of metals levels in tissues of some invertebrate and fish species were observed during summer months that were related to the increased metabolism due to high temperature (Ali and Abdel-Satar, 2005). According to WHO (1984) and FAO (1992) they reported that the permissible limits for Cu, Cd, and Pb in aquaculture water were 1, 0.1 and 0.01 mg/L respectively.

CONCLUSIONS

This study suggested that environment play vital role in the fish health status and its suitability for human use. Fish responds with great sensitivity to changes in the aquatic environment. This study revealed that the levels of heavy metals in all samples were within the acceptable limit. Heavy metals like iron (Fe), copper (Cu), lead (Pb) and cadmium (Cd) were tested in different organs like gills, and flesh tissues of the fish enduring in natural water system. It is obvious that, the levels of all heavy metals detected in water samples and *S. aurata*, *D. labrax* and *A. regius* muscle tissues (the edible portion of fish) were under the notified permissible limits. Some of the measured parameters slightly exceeded the permissible limits recommended by regulatory agencies. It is recommended to treat different wastes before discharging to the natural water sources to avoid the negative effects of pollutants on aquatic life.

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بعض الدراسات الفسيولوجية والبيوكيميائية على تربية بعض الأسماك البحرية في المزارع السمكية لقناة السويس

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¹ قسم الإنتاج الحيواني والثروة السمكية، كلية الزراعة، جامعة قناة السويس، ٤١٥٢٢ الإسماعيلية، مصر
² قسم فسيولوجيا الأسماك، المعمل المركزي لبحوث الثروة السمكية، العباسية، أبو حماد، الشرقية، مصر

تم تنفيذ العمل الحالي لتوضيح تأثير جودة المياه في المزارع السمكية الخاصة في منطقة قناة السويس ومصدر إمداد مياه الصرف الزراعي (منطقة أ، ب)، وتجميع عينات من ثلاثة أنواع مختلفة من الأسماك: الدنيس والقاروص واللوت تم جمعها عشوائياً من ثلاثة قطاعات (ج، د، هـ) تم قياس التغيرات البيوكيميائية والفسيولوجية للأسماك شهرياً على مدار العام من أكتوبر ٢٠١٨ إلى سبتمبر ٢٠١٩ وتم التعامل معها موسمياً في وقت لاحق. على الحالة الفسيولوجية للأسماك، وصورة الدم، والكيمياء الحيوية، وتراكم المعادن الثقيلة في عضلات وخياشيم أسماك الدنيس والقاروص واللوت. وقد وجد أن درجة حرارة المياه في مختلف قطاعات المزارع السمكية على مدار العام كانت معنوية، ومع ذلك، كان الرقم الهيدروجيني متماثل بعض الشيء. على الرغم من أن الأكسجين المذاب في الماء كان حول الحدود المناسبة، فقد تم تسجيل الحد الأدنى له في قطاع المزارع السمكية (ج). ومع ذلك، تم تسجيل أعلى مستويات الملوحة وإجمالي الأملاح الذائبة في هذه المزرعة السمكية. وبالمثل، تم الكشف عن تركيزات الأمونيا والنترت والنترات في هذه المزرعة السمكية. كانت السيادة النسبية للمعادن الثقيلة في مياه الأحواض في قطاعات المزارع السمكية الثلاثة المدروسة طوال فترة الدراسة هي الحديد، والنحاس، والرصاص، والكاديوم. ومع ذلك، فإن المعادن الثقيلة تتراكم بشكل كبير في لحم الأسماك في جميع قطاعات المزارع السمكية أكثر مما تتراكم في عمود الماء. لا يتجاوز المحتوى العضلي للمعادن الثقيلة المكتشفة والتي تتراكم في عضلات وخياشيم جميع أنواع الأسماك التي تم فحصها القيمة المسموح بها التي اقترحتها منظمة الأغذية والزراعة / منظمة الصحة العالمية. حقق مؤشر التلوث المعدني أدنى مستوياته في الماء وأنسجة الأسماك. من الواضح أن مستويات جميع المعادن الثقيلة المكتشفة في عينات المياه وأنسجة العضلات (الجزء الصالح للأكل من الأسماك) كانت أقل من الحدود المسموح بها. بعض الدلائل المقاسة تجاوزت قليلاً الحدود المسموح بها التي أوصت بها الهيئات التنظيمية.



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