Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 25(1): 195 – 211 (2021)
www.ejabf.journals.ekb.eg

Density Effect of the Keeled Mullet Fish *Liza Carinata* on Some Reproductive Hormones and Some Heavy Metals With a Vision of the Mullet Production in Suez Bay.

Mohamed Wafeek[1]; Haytham A. Abd El-Ghaffar[1,*]; Eman M. Zaki[1] and Ahmed F. Makkey[2]

1. Hatchery and Fish Physiology Department, Central Laboratory for Aquaculture Research, Agriculture Research Center, Ministry of Agriculture, Egypt.
2. Population Dynamics Lab. Fisheries Division- National Institute of Oceanography and Fisheries. NIOF. Egypt.

*Corresponding Author:
Dr_haytham1983@hotmail.com

ARTICLE INFO

Article History:
Received: Dec. 19, 2020
Accepted: Jan. 4, 2021
Online: Jan. 21, 2021

Keywords:
Testosterone, growth hormone, cortisol, haematology, heavy metals, keeled production.

ABSTRACT

A total number of 200 *Liza carinata* fish of initial weight about 10g were studied. They were divided by ratio to four densities 50, 100, 150, and 200 fish per cubic meter in aquaria within a wet laboratory for 45 days. Fish have been fed on commercial diets 32% crude protein, twice daily for 6 days per week, as 3% of biomass weight of fish. Total testosterone hormone, FSH, growth hormone, cortisol, haematological parameters, heavy metal in fish gut, muscles, and liver and biochemical composition of fish body were measured at the end of the experiment. Water quality was measured biweekly during the experimental period. Results showed that FSH and testosterone hormones were significantly increased (0.96±0.02 IU/ml and 2.85±0.04 ng/dl**)** in the first treatment for females and males, respectively. Growth hormone was significantly decreased in (T4) which recorded 0.57±0.03 ng/ml. Cortisol was significantly increased (70.5±1.3 µg/dl) in (T4), while it was significantly low (56.3±0.9 µg/dl) in the first treatment. Heavy metals analysis in tissues showed that accumulation of Fe was highest then Zn and finally Cu. Chemical body analysis showed a significant increase for crude protein, crude fat, and ash content in (T1), that recorded (15.6±0.2, 4.22±0.05, and 5.86±0.09 %) respectively. Field visits were performed that revealed the economic, nutritional importance, and productivity of mullet fish in the Suez Bay. It was clear that production is in a continuous decrease throughout the last years.

INTRODUCTION

The Gulf of Suez is the northeastern part of the Red Sea. Suez Bay is the northern part of the Gulf of Suez and provides important nursery areas, feeding sites, spawning grounds, and shelter for many fish species (**Abd EL-Naby *et al.*, 2018**). **Saber *et al.* (2020**) recorded that the Small-Scale Fisheries (SSF) are considered the major economic

activities along the Gulf of Suez and depends mainly on gill nets and trammel nets with different mesh sizes.

The mullet species are of excessive commercial importance in the Egyptian market, and they are highly requested by the customer because of their acceptable quality and prices. Fish production and health are affected by many factors; the most important of them is heavy metal pollution (**Mehanna and Abd El-Azim, 2018**). Mullet contributes about 0.31% of the annual production of the Red Sea, along with GAFRD, 2017 explained by **Abd El-Ghaffar *et al.* (2020**

Reproductive hormones and environmental factors are mainly responsible for the regulation of the seasonal gonadal cycle (**Das, 2011**). In fish, FSH is commonly important for early gonadal development and vitellogenesis (**Aizen *et al*., 2007**). Growth hormone (GH) is one of the polypeptide hormones secreted by somatotrophs in the anterior parts of the pituitary glands of vertebrates, it is involved in the regulation of body growth and maintenance of protein, lipid, carbohydrate, and mineral metabolisms (**Darvish Bastami *et al.*, 2009**). Cortisol, secreted by the internal cells of the head kidney, is a potent gluco and mineralocorticoid in teleostean fish, it plays an essential role in the stress response and osmoregulatory processes (**Flik *et al*., 2006; Arjona *et al*., 2008**).

Haematological and biochemical parameters are being used as a guide in the measurement of health conditions and toxicological signs of organisms (**Rao, 2006**). While providing information about the health status of organisms, these parameters may also indicate exposure to abnormal environmental conditions (**Elahee and Bhagwant, 2007**). Studies of blood parameters had established to be a valuable method for analyzing the health status of fish and help in understanding the relationship of blood features to the habitat and flexibility of the species to the environment (**Bahmami *et al.*, 2001; Fazio *et al.*, 2012**). Several factors such as sex, age, reproductive cycle, nutrition behavior, nutritional status, stress conditions, and water quality, in addition to the habitat of species, cause differences in blood parameters in fish (**Leonardi and Klempau, 2003; Lim and Klesius, 2003; Cnaani *et al*., 2004**). So, it is essential to examine them for a more detailed estimation of fish health status.

Heavy metals are natural trace components of the aquatic environment, but their levels have improved due to industrial, agricultural, and mining activities. As a result, aquatic animals are exposed to high levels of heavy metals, which accumulate in fish's tissues with different magnitudes. Generally, metals accumulation in muscle usually be lower than in gills (**Stancheva *et al.*, 2013**). Since heavy metals are not biodegradable, they could enter the aquatic food chain (**Tuzen, 2003; Altındag and Yig, 2005**) and consequently accumulate in organisms of various trophic levels. Consequently, cause health problems in individuals who consume contaminated seafood (**Dural, *et al.*, 2007**).

This work aimed to study the effect of different fish densities of keeled mullet (*Liza carinata*) on some reproductive hormones, some heavy metals accumulation in tissues, haematology, and chemical body composition. Production of keeled mullet in the Suez Gulf will be investigated.

MATERIALS AND METHODS

This study was carried out on the Keeled mullet, *Liza carinata*. It was divided into two parts; the first part inside the wet laboratory of the National Institute of Oceanography and Fisheries-Suez branch (NIOF) and Central Laboratory for Aquaculture Research (CLAR), and the second part in the Suez Gulf.

1. First part:

In the wet laboratory of the national institute of oceanography and fisheries-Suez branch, a total number of 400 fish of initial weight about 10g were used as a stock. After acclimation for two weeks in fiber containers, fish were divided into four treatments (T1, T2, T3, and T4), in aquaria (each aquarium 40wX50LX60h Cm), depending on ratio densities (50, 100, 150 and 200 fish per cubic meter). T1 was in four replicates, each with 5 fish, T2 was in four replicates, each with 10 fish, T3 was in four replicates, each with 15 fish, and T4 with four replicates, each with 20 fish. Fish were fed commercial diets 32% crude protein twice daily for 6 days per week, as 5% of the fish biomass weight.

At the end of the experiment the following parameters were measured; Follicle Stimulating Hormone (FSH) and testosterone hormones (for both males, and females), growth hormone, cortisol, haematological parameters, and heavy metal in (fish gut, muscles, and liver). Also, water quality was measured every two weeks during the experiment.

1.1. Haematological and hormonal parameter

Fish were anesthetized by MS-222 then blood samples were collected from the caudal veins at the end of the experimental period and divided into two portions. The first part was collected in tubes containing EDTA for haematological parameters determination. Haemoglobin was estimated by the colorimetric method using Boehringer Mannheim Kit as described by **Vankampen (1961).** The count of RBC was determined by taking 0.5 ml of blood containing the anticoagulant EDTA and counted using a double hemocytometer under the microscope as described by **Dacie and Lewis (1991)** and expressed in million per cubic ml. Hematocrits (Hct) value was measured by centrifuging the blood containing EDTA in the micro-hematocrit tube, at 5000 rpm for five minutes until the blood corpuscles were separated from the plasma (**Britton, 1963**). Blood indices also were calculated according to **Houston (1990**) as follows: mean corpuscular volume

(MCV) (fl) = 10. Hct / RBCs ($X10^6$/µl). mean corpuscular haemoglobin (MCH) (Pg) = 10. Hb / RBCs ($X10^6$/µl). mean corpuscular haemoglobin concentration (MCHC) (g/dl) = 100. Hb / Hct.

The second part of the blood was collected within clean and dry tubes then centrifuged for 15 minutes at 5000 rpm. The blood serum was separated, labeled, and frozen with liquid nitrogen for blood biochemical analysis. Testosterone was measured by radioimmunoassay using the procedure described by **Rinchard *et al.* (1993**). FSH hormone was measured as described by **Levavi-Sivan *et al.* (2006)**. The serum GH levels were measured using enzyme-linked immunosorbent assay (ELISA) according to the method of **Drennon *et al.* (2003)** and **Li *et al.* (2010**). Plasma cortisol determination was measured using a validated and characterized radioimmunoassay (**Pickering *et al.*, 1987**).

1.2. Heavy meatal analysis:

Fish were dissected after the experimental period. Parts from gills, liver, and muscles were collected for heavy metal analysis. About 5g from wet organ was dried, ignited, and digested with concentrated HNO_3 and HCL (**AOAC, 2003).** The digested tissues were analyzed for Iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb) by atomic absorption spectrophotometer (Thermo Electron Corporation S series AA spectrophotometer).

1.3. Chemical composition of fish:

Fish's whole bodies were weighed and frozen till analysis. Moisture, ash, protein, and fat estimation for fish samples were done according to **AOAC (2003)**.

1.4. Water quality:

Water parameters were measured biweekly as follows, Temperature and dissolved oxygen using oxygen-meter AQUA LYTIC (model OX 24) instrument. pH was estimated using portable pH electronic paper apparatus "Hanna instruments. Salinity by portable salinity-conductivity meter Lovibond Sensodirect "model, Con 200" UK. Nitrite, nitrate, and ammonium concentration were measured by methods reported by **APHA (2000**).

2. The second part:

2.1. Study area.

Gulf of Suez is a semi-closed area of length that reaches 250 km, with an average width of 54.2 km, and an average depth of 40m. Its total surface area of about 10.510 km^2. The study area extends from the Suez Bay at the north to about 30 Km down the Gulf of Suez. The Suez Bay is the entrance of the Red sea and is limited by longitudes 32^o 28\ and 32^o 35\ E and latitudes 29^o 53\ and 29^o 57\ N **(fig. 1)**.

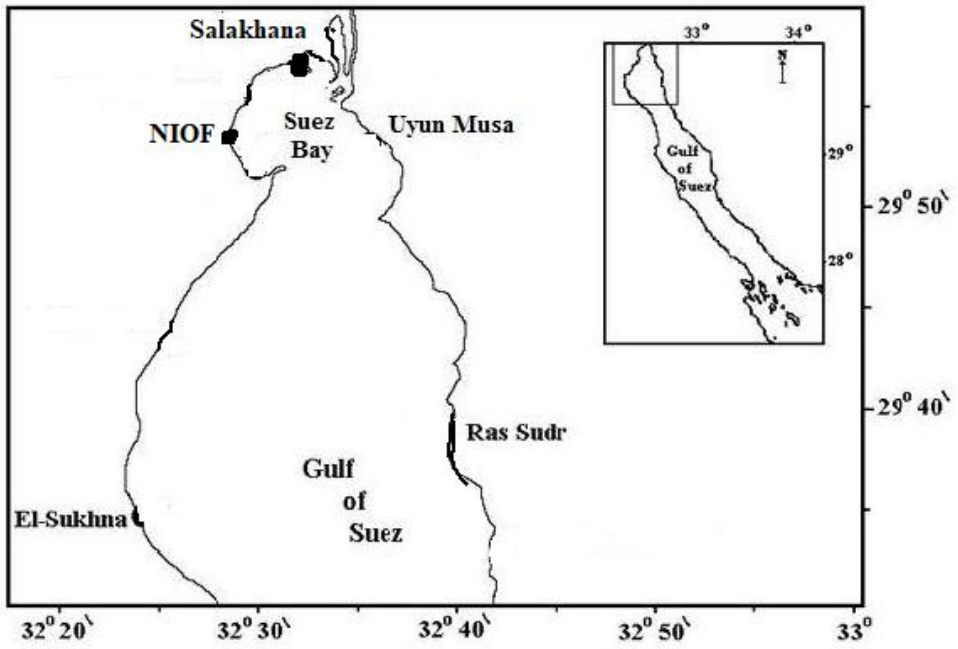

Fig. 1. Location of the study area.

2.2. Fish production:

Through interviews with the traders and fishermen in landing site Salakhana during (September 2019 to September 2020). The mullet catch per fishing ground in the Suez Bay during the last 9 years ago were obtained from fisheries statistical annual reports of the General Authority for Fish Resources Development (**GAFRD, 2018**).

Statistical Analysis:

The obtained data of fish were exposed to one-way ANOVA. Differences between means were tested at the 5% probability level using Duncan test (**Duncan, 1955**). All the statistical analysis was done using SPSS version 20 (SPSS, Richmond, USA) as described by **Dytham (1999)**.

RESULTS

Average means (means±S.D) of some hormones in the blood of Keeled Mullet fish (FSH IU/ml, total testosterone ng/ml, growth hormone ng/dl, and cortisol µg/dl) at the end of the experimental period for different treatments were shown in table (1). FSH hormone values were significant. Treatment (1) showed lowest and highest FSH values 0.22±0.02 and 0.96±0.02 IU/ml for males and females respectively, while its highest and lowest values for males (0.35±0.02 IU/ml) and females (0.59±0.01 IU/ml) were measured in (T4). Total testosterone values were significant, showing highest and lowest values for males (2.85±0.04 ng/dl) and females (0.48±0.01 ng/dl) in (T1). Treatment (4) showed the lowest and highest testosterone values were 2.15±0.06 and 0.69±0.02ng/ml for males and

females respectively. Growth hormone values were significantly decreased in T3 and T4, its highest value 0.91±0.02ng/ml was detected in T1 and the lowest value 0.57±0.03ng/ml were measured T4. Cortisol values were significant among the four treatments, showed the lowest and highest values in T1(56.3±0.9 µg/dl) and in T4 (70.5±1.3 µg/dl), respectively.

Table 1. Average means (Means ± S.D) of some hormones in blood of Keeled Mullet fish (FSH IU/ml, testosterone ng/dl, growth hormone ng/ml and cortisol µg/dl) at the end of the experimental period for different treatments.

	Sex	T1	T2	T3	T4
FSH (IU/ml)	**Males**	0.22±0.02^{c}	0.26±0.01^{b}	0.29±0.02^{b}	0.35±0.02^{a}
	Females	0.96±0.02^{a}	0.76±0.03^{b}	0.68±0.03^{c}	0.59±0.01^{d}
T. Testosterone (ng/dl)	**Males**	2.85±0.04^{a}	2.73±0.07^{b}	2.46±0.08^{c}	2.15±0.06^{d}
	Females	0.48±0.01^{d}	0.55±0.02^{c}	0.63±0.02^{b}	0.69±0.02^{a}
Growth hormone (ng/ml)	**Random**	0.91±0.02^{a}	0.88±0.05^{a}	0.77±0.03^{b}	0.57±0.03^{c}
Cortisol (µg/dl)	**Random**	56.3±0.9^{d}	59.2±1.1^{c}	66.9±1.2^{b}	70.5±1.3^{a}

***Means have the same letter in the same row for the same parameter are not significant (P>0.05)**

Average means (Means ± S.D) of some haematological parameters of Keeled Mullet fish (haemoglobin g/dl, RBCs X10^{6}/cmm, hematocrit %, MCV fl, MCH pg, and MCHC g/dl) at the end of the experimental period for different treatments were shown in table (2). Haemoglobin decreased significantly with increasing fish density. Its highest value 7.9±0.1 g/dl was recorded in the first treatment, while the lowest value 6.7±0.2 g/dl was in T4. Erythrocytes count had a significant difference, the highest count was observed among the first treatment (2.765±0.015 X10^{6}/cmm), and the lowest one (2.345±0.026 X10^{6}/cmm) was noticed among treatment 4. Hematocrits ratio ranged from 19.2±0.6 % in T4 to 22.9±0.2% in T1. The highest MCV value 84.96±0.8 fl was recorded in treatment 3, while its lowest value 81.87±0.9 fl was recorded in treatment 4. Treatment 3 had a significant difference in MCH value which recorded the highest value 29.41±0.3 pg. while treatments (1, 2, and 4) had no significant difference. MCHC had no significant difference and ranged from 34.49±1.4 g/dl in T1 to 34.89±1.1 g/dl in T4.

Table 2. Average means (means±S.D) of some haematological parameters of Keeled Mullet fish (haemoglobin g/dl, RBCs $X10^6$/cmm, hematocrit %, MCV fl, MCH pg and MCHC g/dl) at the end of the experimental period for different treatments.

	T1	T2	T3	T4
Haemoglobin (g/dl)	7.9 ± 0.1^a	7.7 ± 0.1^a	7.2 ± 0.2^b	6.7 ± 0.2^c
RBCs ($X10^6$/cmm)	2.765 ± 0.015^a	2.695 ± 0.019^b	2.448 ± 0.022^c	2.345 ± 0.026^d
Haematocrit (%)	22.9 ± 0.2^a	22.3 ± 0.3^b	20.8 ± 0.4^c	19.2 ± 0.6^d
MCV (fl)	82.82 ± 1.2^b	82.74 ± 0.9^b	84.96 ± 0.8^a	81.87 ± 0.9^b
MCH (pg)	28.57 ± 0.3^b	28.57 ± 0.4^b	29.41 ± 0.3^a	28.57 ± 0.5^b
MCHC (g/dl)	34.49 ± 1.4^a	34.52 ± 1.6^a	34.61 ± 1.3^a	34.89 ± 1.1^a

***Means have the same letter in the same row for the same parameter are not significant (P>0.05)**

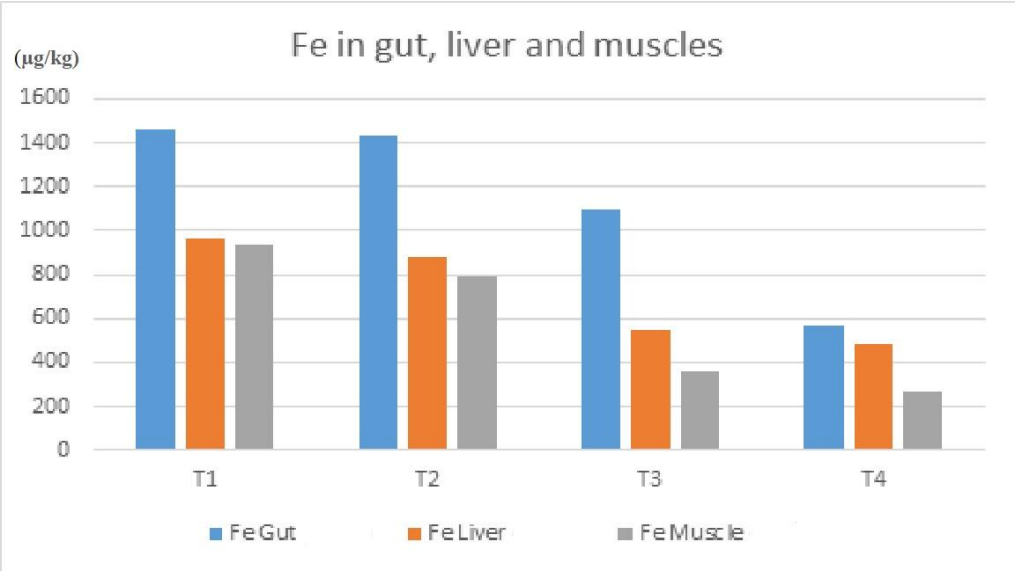

Fig. 2. Iron (Fe) in gut, liver and muscles of *Liza carinata* after the experimental period.

Some heavy metals (iron, zinc, copper, cadmium, and lead) were measured in different fish organs (gut, liver, and muscles) after the experimental period. Data were shown in **fig.** (2, 3, and 4). Cadmium and lead concentrations were too low that couldn't be detected. Iron concentrations in different organs were significant, showing its highest values (1460±12, 960±23, and 935±35µg/kg) for gut, liver and muscle, respectively in T1, while its lowest values for the same organs (562±28, 488±28, and 266±27 µg/kg ,respectively) were determined in T4. Zinc highest concentrations for gut, liver and muscle (113±8, 83±6, and 60±4 µg/kg, respectively) were found in T1, while its lowest values for the same organs (75±6, 33±9, and 43±5 µg/kg, respectively) were determined in T4. Copper highest significant concentrations were obtained in T1 from gut (47±5 µg/kg) and from liver (46±3 µg/kg), while its lowest values for the same organs

respectively (12±3 and 7±3 µg/kg) were determined in T4. Copper values in muscles were non-significant for (T2, T3, and T4), while its significant highest value was obtained in T1 (15±2µg/kg).

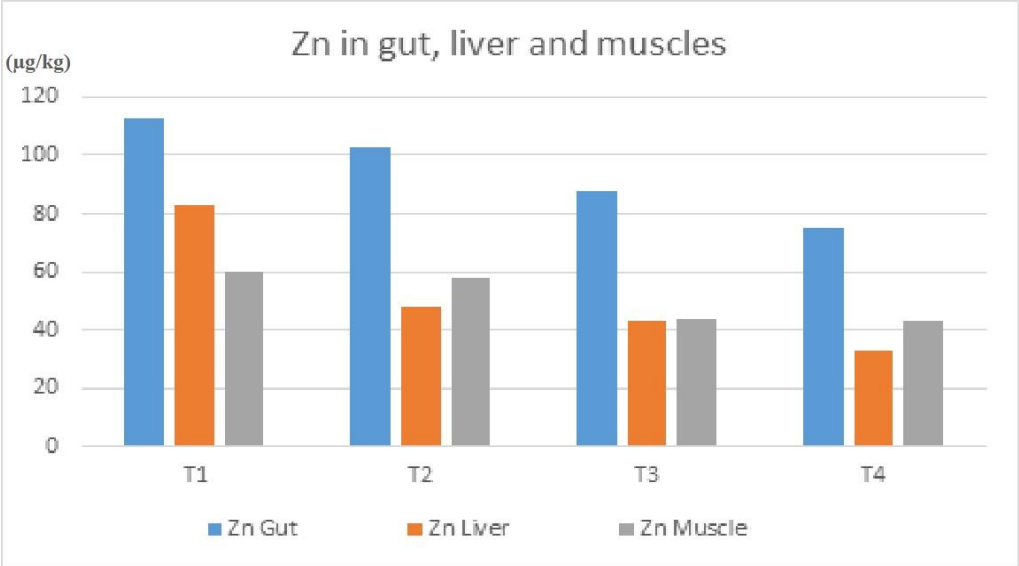

Fig. 3. Zink (Zn) in gut, liver and muscles of *Liza carinata* after the experimental period.

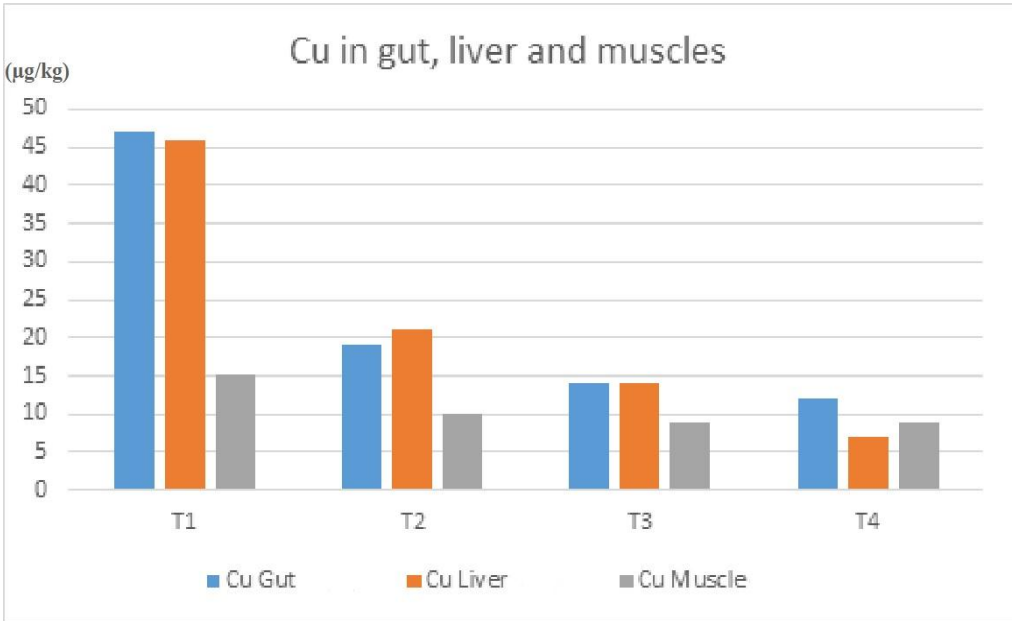

Fig. 4. Copper (Cu) in gut, liver and muscles of *Liza carinata* after the experimental period.

Average means (means±S.D) of some biochemical parameters of Keeled Mullet fish (moisture %, crude protein%, crude fat %, and ash %) at the end of the experimental period for different treatments were shown in table (3). The fish body moisture ratio was

increased significantly from 74.2±0.3% to 76.1±0.3% in T1 and T4 respectively. Crude protein ratio was significantly decreased with increasing fish density showed its minimum ratio14.4±0.1% in T4 and a maximum value 15.6±0.2% in T1. Crude fat ratios were significantly decreased with increasing fish density showing its maximum value of 4.22±0.05% in T1 and minimum ratio 3.82±0.08 in T4. Ash content was significantly decreased, its highest ratio 5.86±0.09% while its lowest ratio 5.64±0.08% was recorded in the 4th treatment.

Table 3. Average means (means±S.D) of some biochemical parameters of Keeled Mullet fish body (moisture %, crude protein%, crude fat % and ash %) at the end of the experimental period for different treatments.

		T1	T2	T3	T4
Moisture	**(%)**	74.2±0.3^{c}	74.9±0.2^{bc}	75.3±0.4^{b}	76.1±0.3^{a}
Crude protein	**(%)**	15.6±0.2^{a}	15.1±0.2^{b}	14.8±0.2^{b}	14.4±0.1^{c}
Crude fat	**(%)**	4.22±0.05^{a}	4.12±0.04^{b}	4.02±0.03^{c}	3.82±0.08^{d}
Ash	**(%)**	5.86±0.09^{a}	5.82±0.07^{a}	5.81±0.09^{ab}	5.64±0.08^{b}

***Means have the same letter in the same row for the same parameter are not significant (P>0.05)**

Data in Table (4) showed the average means (means±S.D) of some physicochemical parameters of water inside the laboratory (Ph, dissolved oxygen mg/l, temperature ºC, salinity ppt, nitrate mg/l, nitrite mg/l, and ammonia mg/l) during the experimental period for different treatments of Keeled Mullet Fish. Physico-chemical parameters of water were monitored bi-weekly during the study period. pH values were non-significant, its values were in between 7.12 ± 0.53 and 7.74 ± 0.35 in T4 and T3 respectively. Dissolved oxygen showed no significance and its measurements ranged from 5.08 ± 0.26 mg/l to 6.66 ± 0.21mg/l in T4 and T1 respectively. Water temperature was ranged from 26.53 ± 1.52ºC in T4 to 27.27 ± 1.46 ºC in T2. Water salinity values were recorded between 41.12 ± 2.34 ppt to 41.16 ± 3.22 ppt in T3 and T2 respectively. The values of nitrate were ranged from 0.15 ± 0.01mg/l in T1 to 0.21 ± 0.02 mg/l in T4. The concentration of nitrite was ranged from 0.12 ± 0.01mg/l to 0.23 ± 0.02 mg/l in T1 and T4 respectively. Concentrations of total ammonia were ranged from 0.27 ± 0.02mg/l to 0.49 ± 0.02 mg/l in T1 and T4 respectively.

Mullet production:

The Suez Bay has one main landing site; Salakhana. The yield of mullet was composed of at least 4 species. However, almost all yield in the Salakhana of catching composed of L. carinata fish. Where, L. carinata was the most abundant species as it accounted for about 95% of the catch of mullet from the Suez Bay according to what was observed on the field visits also from an interview with traders and fishermen (**fig. 6**). The time series data on the mullet catch showed that there is a fluctuation in the catch during

the last 9 years where only 177 tons were landed in 2010 to 66 tons in 2018 according to **GAFRD (2018)** (**Fig. 5**).

Table 4. Average means (means±S.D) of some physico-chemical parameters of water inside the laboratory (Ph, dissolved oxygen mg/l, temperature ºC, salinity ppt, natrate mg/l, nitrite mg/l and ammonia mg/l) during the experimental period for different treatments of Keeled Mullet Fish.

		T1	T2	T3	T4
PH		7.55 ± 0.42 [a]	7.54 ± 0.61 [a]	7.74 ± 0.35 [a]	7.12 ± 0.53 [a]
Dissolved oxygen (mg/l)		6.66 ± 0.21[a]	6.14 ± 0.16 [b]	5.51 ± 0.12 [c]	5.08 ± 0.26 [d]
Temperature	**(℃)**	26.67 ± 1.36 [a]	27.27 ± 1.46 [a]	26.53 ± 1.42 [a]	26.53 ± 1.52 [a]
Salinity	**(ppt)**	41.13 ± 2.21[a]	41.15 ± 2.51[a]	41.16 ± 3.22[a]	41.12 ± 2.34[a]
NO3	**(mg/l)**	0.15 ± 0.01[b]	0.17 ± 0.01[ab]	0.19 ± 0.01[a]	0.21 ± 0.02 [a]
NO2	**(mg/l)**	0.12 ± 0.01[c]	0.14 ± 0.01[c]	0.18 ± 0.02[b]	0.23 ± 0.02[a]
NH3	**(mg/l)**	0.27 ± 0.02[d]	0.31 ± 0.01[c]	0.38 ± 0.01[b]	0.49 ± 0.02 [a]

***Means have the same letter in the same row for the same parameter are not significant (P>0.05)**

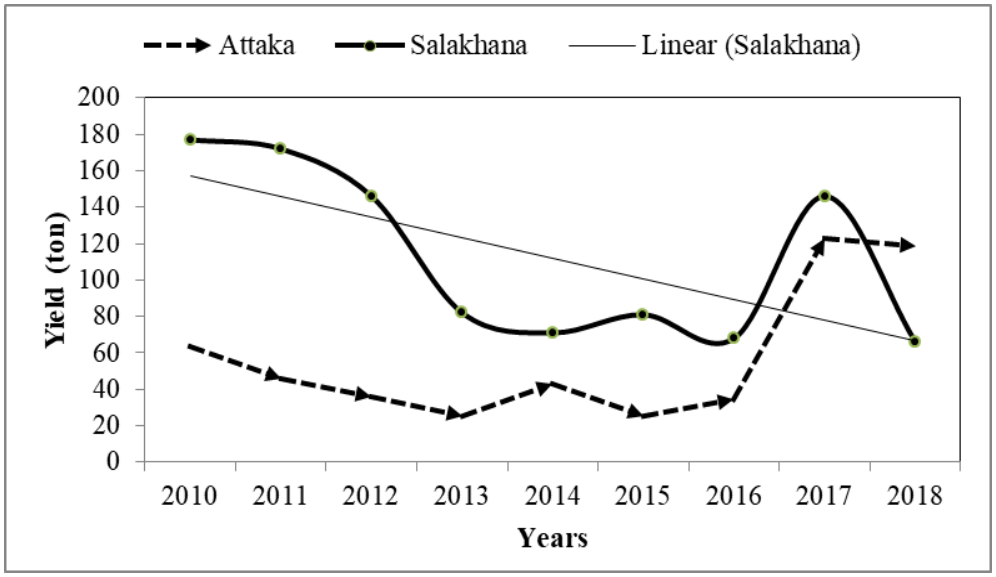

Fig. 5. Comparison between Attaka and Salakhana landing sites of mullet production during nine years ago.

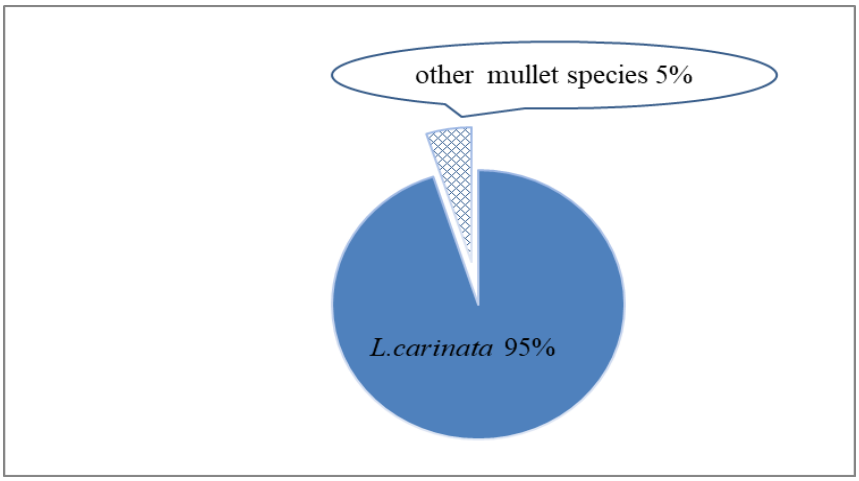

Fig. 6. yield of L. carinata and other mullet species from Salakhana landing site during the period study in Suez Bay.

DISCUSSION

In the present study, FSH was significantly decreased in males from T1, while it was significantly increased in females of the same treatment. Total testosterone was significantly increased in (T1) males and significantly decreased in females of the same treatment. Serum growth hormone (GH) was significantly decreased in (T3 and T4). The serum growth hormone concentration increased with decreasing stocking densities, the present findings are in agreement with those of **Wang *et al*. (2017)** in the African catfish *Clarias gariepinus.* According to **Reading and Sullivan (2017)** follicle-stimulating hormone (FSH) will induce theca cells to release testosterone hormone which in turn stimulates granulosa cells to produce estradiol-17β hormone.

Cortisol is the main adrenocortical hormone of the interregnal gland in teleosts (**McCormick, 2001; Reinecke *et al.*, 2006**), in the mullets, cortisol may also be involved in vitellogenesis and ripening of oocytes because cortisol level was raised in the breeding phase (**Das *et al.*, 2013**). Cortisol may also play some metabolic role in respect of energy manufacture by stimulating glucose creation through gluconeogenesis from amino acid and fatty acids (**Bloom *et al.*, 2000**). Cortisol also causes hyperglycemia in wide varieties of fish (**Zena *et al.*, 2018**). In the present study, cortisol was significantly increased by increasing the fish density which caused fish stress.

Fish density has a negative effect on normal ranges for various blood parameters that have been established by different investigators in fish physiology and pathology (**Darvish Bastami *et al.*, 2009; Satheeshkumar *et al.*, 2011**). Hematological studies help in understanding the association of blood characteristics to the habitat and adaptability of the species to the environment. Several factors cause normal and abnormal differences in

hematologic data (**Clauss *et al.*, 2008)** such as fish species and strain (**Langston *et al.*, 2002**), temperature (**Langston *et al.*, 2002; Magill and Sayer, 2004**), age (**Svetina *et al.*, 2002**), stress (**Cnaani *et al.*, 2004**), photoperiod (**Leonardi and Klempau, 2003**), nutritional state (**Svetina *et al.*, 2002; Lim and Klesius, 2003**), the cycle of sexual maturity, health condition (**Rey Vazquez and Guerrero, 2007**), and water quality. Blood parameters in fishes may be affected by sampling way, analysis procedures, age, habitat, and diet (**Sakamoto *et al.*, 2001**). In the present study, hematological parameters were significantly affected by fish densities as high fish density caused stress on fish leading to decreased the hematological parameters.

In the present study, five metals (Fe, Zn, Cu, Cd, and Pb) were measured in three different organs (gut, liver, and muscles) of experimental fish. Cd and Pb measures were too low that couldn't be read by the instrument. The heavy metals concentration in the measured organs were all lower than permissible values established standard for the aquatic environment by **WHO and FAO (1989)**. The iron accumulation followed the order of gut > liver > muscles, this patteren may be related to the fact that gut is the first receptor for aquatic environmental heavy metals, and the liver is responsible for detoxification while muscles are inactive organs contained the lowest heavy metals concentrations. The lowest values were recorded among T4, where a higher stocking density was used. The accumulation of heavy metals in the measured organs showed that Fe was the highest one followe by Zn while the lowest one was Cu. This result agreed with that obtained by **Mehanna and Abd El-Azim (2018)** in keeled fishs' muscles, but differ for liver, this is may be due to the condition of sampling. High levels of Zn and Cu in liver are always related to a natural binding protein such as metallothioneins (MT) (**Gorur *et al.*, 2012**), which act as a vital metal store (i.e., Zn and Cu) to fulfill enzymatic and other metabolic requirements. liver is the greatest suggested tissue as environmental indicators of water pollution (**Karadede *et al.*, 2004**). Besides, muscle usually exhibited low metal levels, which may be attributed to the fact that muscle is not an active tissue concerning metal accumulation (**Visnjic-Jeftic *et al.*, 2010**). Nevertheless, metal analysis in muscle remains important due to flesh is the part that is used for individuals consumption (**Mehanna and Abd El-Azim, 2018).**

Results showed that the moisture was significantly increased by increasing fish density. The crude protein and crude fats were significantly increased by decreasing the fish density.

According to **Avelar De Carvalho *et al.* (2019**), the parameters of water quality during the larvae culture of mullet *Mugil liza* in the laboratory conditions were within the permissible limits. Water quality parameters in this study were measured biweekly, and the mean measurements were shown in table (4). pH was non-significant, dissolved oxygen was significantly increased by decreasing fish density. Water temperature and

salinity were in the same range for the four treatments. Nitrite, nitrate, and ammonia were significantly increased in (T4) by increasing fish density.

The present results showed that the mullet family production in 2018 was very low compared to the previous year. This is due to several factors (a)- the capture of fry from the Suez Bay, which is one of the most important areas for spawning of mullet fish (especially the Sehlia fish), (b)- the pollution level as this area of the Suez Bay is containing more than one source of pollution according to **Mehanna and Abd El-Azim (2018),** (c)- overfishing. Also, **Holden and William (1974)** mentioned that practically, fisheries are dealing with the data collected to answer two main questions: "How much fish is there in the area that is intended to fish and What is the maximum amount of fish which can be caught annually without affecting the ability of the stock to produce that yield".

CONCLUSION

Fish density negatively affects reproductive hormones, growth hormone, and physiological state of keeled mullet *Liza carinata*, but it has a positive effect on the heavy metals accumulation in different fish organs in the presence of suitable conditions of water quality. It was clear that production was continuously decreasing during the last ten years and therefore, we recommend rationalizing fishing in the Gulf to preserve this economic family and ensure the optimal and sustainable exploitation of it.

REFERENCES

Abd El-Ghaffar, H.A.; Zaki, E.M. and Makkey, A.F. (2020). Effect of Age on Growth of Keeled Mullet Fish *Liza Carinata* (Valenciennes, 1836) in Different Environmental Conditions (Wild And Lab.) Egyptian Journal of Aquatic Biology & Fisheries, 24(7): 863–877.

Abd EL-Naby, A. S.; El-Ganainy, A. A; Mohamed,M. A. and El-Mor, M. (2018). Species composition and size structure of experimental beach sein by-catch in eion moussa, north Gulf of Suez, Egypt. Al Azhar Bulletin of Science 29(2): 39-47.

Aizen, J.; Kasuto, H.; Golan, M.; Zakay, H. and Levavi-Sivan, B. (2007). Tilapia Follicle-Stimulating Hormone (FSH): Immunochemistry, Stimulation by Gonadotropin-Releasing Hormone, and Effect of Biologically Active Recombinant FSH on Steroid Secretion. *Biology of Reproduction*, 76(4): 692–700.

Altındag, A. and Yig, S. (2005). Assessment of Heavy Metal Concentrations in the Food Web of Lake Beys_Ehir, Turkey," Chemosphere, 60(4): 552-556.

AOAC, (Association of Official Analytical Chemist). (2003). Official methods of analysis. 17th ed. Arlington, Virginia.

APHA. (American Public Health Association). (2000). Standard Methods for the Examination of Water and waste water (20thedition). Washington, D.C.

Arjona, F.J.; Vargas-Chacoff, L.; Martín del Río, M.P. Flik, G. Mancera, J.M. Klaren, P.H.M. (2008). The involvement of thyroid hormones and cortisol in the osmotic acclimation of *Solea senegalensis*. General and Comparative Endocrinology, 155: 796-803.

Avelar De Carvalho, C.V; Passini, G.; Sterzelecki, F.C.; Baloi, M.F. and Cerqueira, V.R. (2019). Maturation, spawning and larviculture of the mullet Mugil liza under laboratory conditions. Rev Bras Reprod Anim, 43(1): 31-36.

Bahmami, M.; Kazemi, R. and Donskaya, P. (2001). A comparative study of some hematological features in young reared sturgeongs (Acipenser persicus and Huso huso). Fish Physiol Biochem, 24;135-140.

Bloom, S.; Anderson, T.B. and Forlin, L. (2000) Effects of food deprivation and handling stress on head kidney 17α-hydroxyprogesterone 21-hydroxylase activity, plasma cortisol and the activities of liver detoxification enzymes in rainbow trout. Aquat Toxicol., 48: 265-274.

Britton, C.J. (1963). Disorders of the blood. 9th ed. I. A. Churchill, Ld. London: 42P.

Clauss, T.M.; Alistair, D.M. and Arnold, D.J.E. (2008). Hematologic disorders of fish. Vet Clin N Am Exot Anim Pract, 11;445-462.

Cnaani, A.; Tinman, S.; Avidar, Y.; Ron, M. and Hulata, G. (2004). Comparative study of biochemical parameters in response to stress in O. aureus, O. mossambicus and two strains of O. niloticus. Aquaculture Research 35 (15): 1434-1440.

Dacie, S. and Lewis, S. (1991). Practical hematology, 7th ed., Churchill Livingstone, London: 633.

Darvish Bastami, K.; Haji Moradlou, A.; Mohamadi Zaragabadi. A.; Salehi Mir, S.V. and Shakiba, M.M. (2009). Measurement of some hematological characteristics of the wild carp. Comp Clin Patho. 18(3): 321-323.

Das, P. (2011). Circannual and circadian variation of ovarian activity with associated changes in hormonal and blood glucose profiles and in vitro induction of oocyte maturation and steroidogenesis in an estuarine grey mullet Mugil cephalus L. Ph.D. Thesis, University of Calcutta. Phelps RP. Recent advances in fish hatchery management. Revista Brasileira de Zootecnia, 39: 95-101.

Das, P.; Pramanick, K.; Maity, A. and Maiti, B.R. (2013). The role of some extra-gonadal hormones on the circannual ovarian cycle of the flat head grey mullet, Mugil cephalus L. Biological Rhythm Research, 44:(5): 830-843.

Drennon, K.; Moriyama, S.; Kawauchi, H.; Small, B.; Silverstein, J.; Parhar, I. and Shepherda, B. (2003). Development of an enzyme-linked immunosorbent assay for the measurement of plasma growth hormone (GH) levels in channel catfish (Ictalurus punctatus): assessment of environmental salinity and GH secretogogues on plasma GH levels. Gen. General and Comparative Endocrinology, 133(3), 314-322.

Duncan, D.B. (1855). Multiple range and multiple F test. Biometrics., 11:1-42.

Dural, M.; Goksu, M.Z.L. and Ozak, A.A. (2007). Investigation of Heavy Metal Levels in Economically Important Fish Species Captured from the Tuzla Lagoon," Food Chemistry, 102: (1), pp. 415-421.

Dytham, C. (1999). Choosing and using statistics: a biologist's guide. Blackwell Science Ltd., London, UK.

Elahee, K.B. and Bhagwant, S. (2007). Hematological and gill histopathological parameters of three tropical fish species from a polluted lagoon on the west coast of Mauritius. Ecotox Environ Saf, 68; 361-371.

Fazio, F.; Faggio, C.; Marafioti, S.; Torre, A. and Sanfilippo, M. (2012). Comparative study of hematological profile on Gobius niger in two different habitat sites: Faro Lake and Tyrrhenian Sea. Cahiers de Biologie Marine 53 (2): 213-219.

Flik, G.; Klaren, P.H.M.; Van Den Burg, E.H.; Metz, J.R. and Huising, M.O. (2006). CRF and stress in fish. Gen. Comp. Endocrinol., 146: 36-44.

GAFRD, (General Authority for Fish Resources Development). (2018). Annual report for country fish production in 2018.

Gorur, F.K.; Keser, R.; Akcay, N. and Dizman, S. (2012). Radioactivity and heavy metal concentrations of some commercial fish species consumed in the Black Sea Region of Turkey. Chemosphere; 87:356-61.

Holden, M.J. and William, T. (1974). The biology and population structure of bass, *Dicentrarchus labrax*, in English waters. J. Mar. Biol. Assoc. U.K. 54: 91-107.

Houston, A.H. (1990). Methods for fish Biology. Blood and circulation. Chapter 9: 237-334.

Karadede, H.; Oymak, S.A. and Ünlü, E. (2004). Heavy metals in mullet, Liza abu, and cat-fish, Silurus triostegus, from the Atatürk Dam Lake (Euphrates), Turkey. Environ.Int. 30, 183–188.

Langston, A.L.; Hoare, R.; Stefansson, M.; Fitzgerald, R.; Wergeland, H. and Mulcahy, M. (2002). The effect of temperature on non-specific defence parameters of three strains of juvenileAtlantic halibut (Hippoglossus hippoglossus L.). Fish Shellfish Immun, 12; 61-76.

Levavi-Sivan, B.; Biran, J. and Fierman, E. (2006). Sex steroids are involved in the regulation of gonadotropin-releasing hormone and dopamine D2 receptors in female tilapia pituitary. Biol Reprod, 75: 642–650.

Leonardi, M.O. and Klempau, A.E. (2003). Artificial photoperiod influence on the immune system of juvenile rainbow trout (Oncorhynchus mykiss) in the Southern Hemisphere. Aquaculture 221 (1-4): 581-591.

Li, Y.P.; Diao, X.M.; Sheng, X.S.; Quan, H.; Zhai, X.L. and Li, Y. (2010). Purification and characterization identification of vitellogenin from pelteobagrus vachelli (in Chinese, English abstract). Oceanologia et Limnologia Sinica, 41(1), 91-98.

Lim, C. and Klesius, P.H. (2003). Influence of feed deprivation on hematology, macrophage chemotaxis, and resistance to Edwardsiella ictaluri challenge of channel catfish. Journal of Aquatic Animal Health 15 (1): 13-20.

Magill, A.H. and Sayer, M.D.J. (2004). The eject of reduced temperature and salinity on the blood physiology of juvenile Atlantic cod. J Fish Biol 2004;64;1193-1205.

McCormick, S.D. (2001). Endocrine control of osmoregulation in teleost fish. Am Zool., 41:781-794.

Mehanna, S.F. and Abd El-Azim, H.M. (2018). Seasonal Variation of heavy metals in Water, Sediment and Liza carinata from the northern Gulf of Suez, Egypt and its effect on mullet production and growth rate at the Gulf. 3rd International Conference on Integrated Environmental Management for Sustainable Development: 44-53.

Pickering, A.D.; Pottinger, T.G. and Sumpter, J.P. (1987). On the use of dexamethasone to block the pituitary-interrenal axis in the brown trout, Salmo trutta L. Gen. Comp. Endocrinol. 65: 346-353.

Rao, J.V. (2006). Biochemical alterations in euryhaline fish, *Oreochromis mossambicus* exposed to sub-lethal concentrations of an organophosphorus insecticide, monocrotophos. Chemosphere 2006; 65; (10.);1814 -20.

Reading. B.C. and Sullivan, V.C. (2017). Vitellogenesis in Fishes. North Carolina (US): Elsevier Inc. hlm 1–12.

Reinecke, M.; Zaccore, G. and Kapoor, B.G. (2006). Fish endocrinology. Vol. 1. Enfield: Science.

Rey Vazquez, G. and Guerrero, G.A. (2007). Characterization of blood cells and hematological parameters in Cichlsoma dimerus. Tissue Cell, 39:151-160.

Rinchard, J.; Kestemont, P.; Kuhn, E.R. and Foster, A. (1993). Seasonal changes in plasma levels of steroid hormones in an asynchronous fish the gudgeon Gobio gobio L; (Teleosti, Cyprinidae), Gen. Comp. Endocrinology, 92: 168-178.

Saber M. A., Shaaban A. M., El ganainy A. A. and Osman H. M. (2020). Species composition, length-weight relationships and condition factor of commercial species grasped by trammel nets and gill nets in the Gulf of Suez, Red Sea, Egypt. Egyptian Journal of Aquatic Biology & Fisheries, 24(3): 145 – 156.

Sakamoto, K.; Lewbart, G.A. and Smith, T.M. (2001). Blood chemistry values of juvenile red pacu (*piaractus brachypomus*). Vet Clin Pathol, 30: 50-52.

Satheeshkumar, P.; Ananthan, G.; Senthil Kumar, D. and Jagadeesan, L. (2011). Hematology and biochemical parameters of different feeding behaviour of teleost fishes from Vellar estuary, India. Comp clin Patho 2011.

Stancheva, M.; Makedonski, L. and Petrova, E. (2013). Determination of heavy metals (Pb, Cd, As and Hg) in Black Sea grey mullet (Mugil cephalus). Bulg. J. Agric. Sci., Supplement 1: 30–34

Svetina, A.; Matasin, Z.; Tofant, A.; Vucemilo, M. and Fijan, N. (2002). Hematology and some blood chemical parameters of young carp till the age of three years. Acta Vet Hung, 50 ;459-467.

Tuzen, M. (2003). Determination of Heavy Metals in Fish Samples of the Middle Black Sea (Turkey) by Graphite Furnace Atomic Absorption Spectrometry," Food Chemistry, 80: (1), pp. 119-123.

Vankampen, E.J. (1961). Determination of hemoglobin. Clin. Chem.. acta., 6:538-544.

Visnjic-Jeftic, Z.; Jaric, I.; Jovanovic, L.; Skoric, S.; Smederevac-Lalic, M.; Nikcevic, M. and Lenhardt, M. (2010). Heavy metal and trace element accumulation in muscle, liver and gills of the Pontic shad (*Alosa immaculata* Bennet 1835) from the Danube River (Serbia). Microchem. J. 95, 341–344.

Wang, X.M.; Gao, J.W.; Xu, M.; Mo, B.L.; Dai, W. and Chen, C.H.X. (2017). Responses of growth rates and growth hormone levels of African catfish (*Clarias gariepinus*) to stocking density. Iranian Journal of Fisheries Sciences. 16(2): 698-710

WHO/FAO (World Health Organization/Food and Agriculture Organization of theUnited Nations). (1989). Heavy Metals – Environmental Aspects. Environment Health Criteria. No. 85, Geneva, Switzerland.

Zena, L.A.; Dillon, D.; Hunt, K.E.; Navas, C.A.; Bícego, K.C. and Buck, C.L. (2018). Seasonal changes in plasma concentrations of the thyroid, glucocorticoid and reproductive hormones in the tegu lizard *Salvator merianae,* General and Comparative Endocrinology.