

## **Study on clinicopathological and biochemical changes in some Marine water Fishes infested with internal parasites in Red Sea.**

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### **Abstract**

Helminthology and haematology parameters have been recognized as the important tools for assessing the fish health. Measurement of blood parameters has been used for many years as a tool for monitoring the health of fish. The present study investigated the effects of a natural infestation of *Siganus rivulatus*, *lethrinus harak* and *Gerras oyena* with internal parasites on blood parameters such as red blood cell (RBCs) and white blood cells counts (WBCs) and hemoglobin level were estimated as well as plasma glucose and serum activities of liver and kidneys function tests. Our study was conducted on 275 marine fish 100 of *Siganus rivulatus*, 100 of *lethrinus harak* and 75 of *Gerras oyena* collected from water of Safaga city at Red Sea governorate for examination of internal parasitic infestation. Parasitized fish showed decreases RBCs level ( $1.41 \times 10^6/\mu\text{L}$ ), low hematocrit (17.82%) and increases in WBCs ( $18.37 \times 10^3/\mu\text{L}$ ) and increases in plasma glucose levels (68.4 mg/dl), while decreased in serum total proteins (2.81 g/dl). AST activities in serum (95.1 U/L), ALT activities in serum (81.65 U/L) and Creatinine value (1.10 mg/dl) all increases compared with values in healthy non infected fish due to increase of helminthes infection. Decrease in serum urea (24.25 mg/dl) level was also found. The result revealed that fish were apparently healthy and no pathgnomic signs of infested fish except excessive mucous secretion. In case of P. M. examination of infested fish liver there was paleness with areas of hemorrhage and somewhat Congestion with peticeal hemorrhage, inflammation of Intestine. It was 46.0% of examined fish infested with *Nematodes spp.* were recorded. The highest percentage of infestation with *Nematode spp.* was more than 150 g. and 25 cm. in case of *Siganus rivulatus*, 14. % in *Lethrinus harak*. and 60% in *Gerras oyena*.

**Key words:** hematological; marine fish; Fish parasite; liver function and kidney function tests

### **Introduction**

Fish is an essential component of daily diet of many people in Egypt. Egypt is topographically situated along side great areas of fresh and salt water and is one of the coastal countries that must take benefit from fish proteins. Fish is an excellent protein source (**Jannat et al., 2010**) and provides many health benefits. Normally fish muscle is sterile as its immune system prevents bacteria to proliferate easily whereas after death the fish's immune system collapses allowing easily penetration of microorganisms into the

flesh, **(Huss, 1995)**. Like human and other animals, fish suffers from diseases and infested with parasites. In the same time, the internal parasitic diseases have the upper hand in fish parasitic diseases regarding the low body gain, high mortality, marketability and some of these diseases may have zoonotic importance **(Eissa, and Hala, 1993 and Eissa, 2002)**. Most nematodes present in the alimentary system and only few enter tissues or inner cavities **(Paperna, 1996)**. Parasitic infestions often give an indication of the quality of water, since parasites generally increase in abundance and diversity in more polluted waters **(Poulin, 1992; Noga, 2010)**. Parasites are capable to cause damage to the fish through injury to the tissues or organs. Roundworms called nematodes are the most common parasites found in marine fish **(Hilderbrand *et al.*, 1985)**. Fish defenses against diseases are specific and nonspecific, where non-specific defenses include skin and scales, as well as the mucus layer secreted by the epidermis that traps microorganisms and inhibits their growth. If pathogens breach these defenses, fish can develop inflammatory responses that increase the flow of blood to infected areas and deliver white blood cells that attempt to destroy the pathogens. According to previous records, blood parameters serve as reliable indicators of fish health as many parasites can live in a fish, where fishes died due to their toxicity **(Bond, 1979)**. Therefore, the changes associated with hematological parameters due to various parasites can serve important information which could be used for disease diagnosis and guidelines for the implementation of the treatment in the future. This knowledge would be very much helpful in fish farming and fish industry **(Roberts, 1981)**. Haematology is considered as one of the key tools to assess the health status index of different species because it is provide a reliable evaluation via non-lethal means **(Satheeshkumar *et al.*, 2012)**. The analysis of blood indices has proven to be a valuable approach for analyzing the health status of fish as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting **(Bahmani *et al.*, 2001)**. The highest rate of parasitic infestation changes the blood parameters spontaneously and blood biochemical factors (blood sugar, hemoglobin and creatinine) revealed significant variation between infested and uninfested fishes **(Marzan *et al.*, 2014)**. Most of the bacteriological studies that have been done on fish concern their external surfaces (skin and gills) or their digestive tracts **(Sugita *et al.* 1985; Sakata, 1989)**. In general, the bacterial communities present on the external surfaces of fishes reflect the bacterial load of the water in which these aquatic vertebrates are living, while the microbial diversity present in the digestive tracts is almost always much lower than in the water. As the spleen, the liver and the kidneys should be sterile in healthy fishes. **Moustafa *et al.*, (2010)** concluded that bacterial pathogens are the most significant microbial agents affecting marine fishes and climatic changes may plays a great role in modulating the occurrence of bacterial fish diseases. So we must examine the fish for

bacteriology besides parasitology to be sure that all alterations in blood come from infestation with parasites only. There are many reports indicate that, evaluation of disturbance caused by parasitism in fishes, depend on the determination of their blood parameters. Therefore, objective of this study is to investigate the impact of internal parasites on some physiological parameters related to liver and kidney functions as well as its effect on fish haemopirotic system of some marine water fish infested with internal parasites.

## **Materials and Methods**

### **1. Fish**

A total number 275, (100 *Siganus revulatus*, 100 of *lethrinus harak* and 75 of *Gerras oyena*) marine fishes were collected a live from Safaga city at Red Sea governorate, then transported a live to the laboratory. First weight and total length of the examined fish were recorded.

### **2. Clinical picture:**

Was done on the live fish or freshly dead ones according to the methods described by (Amlacker, 1970). Fish were grossly examined to investigate any lesions on the external body surface and internally for parasitic infestation according to (Conroy and Hermann, 1981).

### **3. Parasitological examination:**

The examination was performed on the freshly dead fishes. The collected nematodes were washed in physiological saline, after full relaxation fixed in hot alcohol - glycerin 5% until all alcohol evaporated and the specimen remains in nearly absolute glycerin. They were cleared in lacto-phenol and mounted in glycerin-gelatin according to (Meyer and Olsen, 1992), left to dry and examined microscopically.

### **4. Biochemical and hematological examinations:-**

#### **4.1. Hematological examination:**

Two blood samples were collected by caudal venipuncture. One sample was collected with a syringe containing anticoagulant 10%-EDTA and the other without it. These procedures were carried out within 0.8-1.2 minutes to minimize stress. The blood samples containing EDTA were divided into two parts. One part was used to measure plasma glucose according to (Cooper, and McDaniel, 1970) and the other part for determination of red blood cell count (RBC), haematocrit (HCT), and haemoglobin concentration (HB), blood smears stained were used for total white blood cell and differential leukocyte counts were performed according to (Jain, 2000). The second blood sample, without anticoagulant, was left for 30 min. at room temperature and then centrifuged at 3000 rpm for 10 minutes to separate serum and was used later for various

biochemical analyses. The separated serum was frozen at  $-20^{\circ}\text{C}$  until analysis (**Lied *et al.*, 1975**).

#### **4.2. Biochemical examination:-**

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum were determined according to (**Bergmeyer *et al.*, 1986**) and serum total proteins were determined according to the method described by (**Peters *et al.* 1982**). Creatinine value was determined according to (**Rock *et al.*, 1987**). Urea concentration was measured according to (**Pathson and Nauch, 1977**). All these biochemical analyses were measured calorimetrically using spectrophotometer and purchased kits.

#### **5. Bacteriological examinations:-**

Samples from gills, liver, spleen and kidney from fishes were cultured on general and selective media. Then identification of the isolates pure cultures of the isolates were identified by biochemical characterization following the criteria proposed by those described in the Bergey's Manual of Determinative Bacteriology, (**Garrity, 2001**). Final confirmation of each strain was achieved using the analytical profile index of API20-E system (**Buller, 2004**).

#### **6. Statistical analysis:**

The obtained data were statistically analyzed and tested for significance according to (**Petrie, and Waston, 1999**).

### **Results and Discussion**

Fish does not only serve as the host for different parasites but some parasitic forms cause serious damage to the tissue and also alters the normal physiology, histology and haematology of the host. Blood is a good indicator to determine the health of an organism (**Joshi *et al.*, 2002**). It also acts as a pathological reflector of the whole body. Hence, the haematological parameters are important in diagnosing the functional status of the fish (host) infested by helminth parasites (**Joshi *et al.*, 2002**) and also to evaluate the physiological condition and nutritional state of fish (**Chagas and Val, 2003**).

The total body length of examined *Siganus revulatus*, *Lethrinus harak* and *Gerras oyena* fishes was 18-25, 23-29 and 22-27 cm. The body weight from 60 to 400, 160-350, 190-250 g. respectively.

The **clinical signs** of *Siganus revulatus*, *lethrinus harak* and *Gerras oyena* naturally infested with nematodes revealed no pathognomic clinical abnormalities except excessive mucous secretion, which may be used to dilute the irritation and act as a defense mechanism against the infestation this is in agreement with (**Maather, and Heba, 2012**), (**Nashwa, 2010**) and (**Yambot, and Lopez, 1997**).

**Postmortem Examination** of infected fishes with nematodes showing inflammation of Intestine with enlargement as well as heavy infestation of *Nematode.spp*(**Fig. 3**) and paleness of liver with areas of hemorrhage and somewhat Congestion with peticeal hemorrhage, (**Figs. 1, 2**) this is similar to that obtained by (**Yanong, 2006**), (**Eissaet al., 2010**) and (**Mai, 2013**).

**Parasitological examination** of the infested marine fishes revealed that the total infestation rate was 38.0%. This result is lower than that recorded by (**Sarah, 2015**), (72.3%), and higher than that recorded by (**Anthony et al., 2014**), (18.5%), and nearly agree with (**Mohammed et al., 2015**), (35.9%). *Siganus revulatus* the infestation rate by *Nematode spp.* was 46% in the intestine of fish as shown in (**Figs. 4, 5**). This percentage higher than that of (**Eman, and Derwa, 2005**) (4.8%), (**Mai, 2013**) (9.5%) and (**Mo, et al., 2014**) (20%) and lower than that recorded by (**Wafaa, 2012**) (67%). Regarding to *Lethrinus harak* infected with nematode with percentage 14% (**Figs. 6, 7, 8, 9, 10**), this result lower than that of (**Nashwa, 2010**), and in case of *Gerras oyena* the percentage of infestation was 60% (**Figs. 11, 12, 13, 14, 15, 16, 17**). This variation may be attributed to the difference of location from which the fishes and the time of research.

**The Prevalence of the detected parasitic infection** in relation to fish body weight. The highest percentage of infections with *Nematode spp.* in *Siganus revulatus* was in more than 150 g. regarding the length the highest prevalence was in more than 25 cm. It could be related to the continuous exposure of fish during their life stage to cercarial invasions from surrounded water containing high number of infested snails. Also when the length of fish increased this will offer a good chance of exposure to infestation with cercariae. In the case of *Lethrinus harak* and *Gerras oyena* there is no clear relationship between body weight and total length of *Lethrinus harak* and *Gerras oyena* and this due to the environmental factors which may influence the degree of infestation of the hosts as well as the population parameters of parasites and biology of hosts recorded during reproduction. The presence of nematode infestations is determined by environmental conditions which may act both on the worms and the hosts, large agglomerations, food and fish migrations (**Anderson, and Gordon, 1982; Bagge et al., 2004**).

Regarding to the bacteriological examination for investigated fish it was bacteriologically free from infection.

#### **Haematological and Biochemical investigation were:-**

During the course of investigation, of infested and non-infested specimens of fish, the total haemoglobin content, number of erythrocytes, granulocytes and lymphocytes were observed in (**Tab. 1**). And regarding to the hematological and biochemical changes of fish infested with parasites, it was noticed that there was a picture of microcytic hypochromic type of anemia in infected fish, represented by reduction in erythrocytic count ( $1.41 \pm 0.07$ ),

hemoglobin concentration ( $7.93\pm 0.39$ ), packed cell volume (PCV) ( $17.82\pm 0.83$ ), mean corpuscular hemoglobin concentration (MCHC) ( $44.46\pm 2.18$ ) associated with an elevation in mean corpuscular volume (MCV) ( $126.66\pm 5.78$ ) of infested fish compared with non-infested one. These results may be due to that the damage of fish caused by the parasite is considered to be related mainly to the blood-sucking activity of the (pre) adult stages of the nematode in the swim bladder. These because the gut of the adults is completely filled with erythrocytes, so the direct damage done from the blood-sucking of mature worms as well as, changes produced in the swim bladder wall by the migration of larvae and the pathological effects become progressively more pronounced as repeated infections occur (**Haenen et al., 1996**). Also off food of infested fish may cause anemia. The results nearly agree with the results previously recorded by (**Boon et al., 1990**), (**Benajiba et al., 1994**) and (**Dosoky, 2007**). The reduction in RBCs count, Hb value and packed cell volume in *Siganus revulatus* infested with *Nematode spp.* occurred as a result of the parasitic infestation that often leads to anemia (**Martins et al., 2004**). According to (**Lebelo et al., 2001**) and (**Hassen, 2002**) the increase in WBCs ( $18.37\pm 0.45$ ) count occurred as a pathological response since these WBCs play a great role during infestation by stimulating the haemopoietic tissue and immune system by producing antibodies and chemical substances working as defense against infection.

The indices of the liver and kidney function of infested and non-infested fish which showing significant increase in the liver enzyme than normal range. As well as fish showed higher mean values of the indices of kidney function including blood urea and creatinine than the corresponding values of the non-infested fish showed in **Table (2)**. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Creatinine and glucose were higher in infested fish than non-infested one. They were ( $81.65\pm 3.61$ U/l), ( $95.1\pm 4.74$ U/l), ( $1.10\pm 0.02$  mg/dl) and ( $68.4\pm 2.19$  mg/dl) respectively in infested while in non-infested fish were ( $38.74\pm 1.72$  U/l), ( $54.80\pm 2.18$  U/l), ( $0.94\pm 0.01$  mg/dl) and ( $59.4\pm 1.35$  mg/dl) respectively. The increase of serum activities AST and ALT of infested fish is perhaps due to hepatic cells injury and increased release of the enzyme by the liver as clarified by (**Yang, and Chen, 2003**).

Significant hyperglycaemia was observed compared with non-infested fish. A similar finding was reported for *Oncorhynchus mykiss* parasitized by *A. japonicus* (**Ruane et al., 1999**) and *L. salmonis* (**Ruane et al., 2000**). Glucose levels can increase in response to elevated epinephrine and cortisol activity (**Bowers et al., 2000; Haond et al., 2003**). The observed decrease in serum urea ( $24.25\pm 1.57$  mg/dl) level was nearly similar to that recorded by (**Tandron, and Chandra, 1973**) who attributed this decrease in serum urea level to probable inhibition in urea synthesis as a result of hepatocellular damage. The

observed elevation in serum creatinine level could be attributed to the obtained renal dysfunction.

As well as **Table (2)** shows that the serum total proteins ( $2.81 \pm 0.19$  mg/dl) was significantly decreased in heavily infested fish in comparison with non- infested fish which was similar to that described by (**Steinhagen et al. 1997**) and (**Hamouda, 2011**). This decrease may be as a result of consumption of nutrient material by the parasite, also can be resulted from destruction occurred in intestinal mucosa that allow leakage of plasma protein and destruction of intestinal villi which are responsible for absorption of nutrients and protein from food materials. These findings may act as immunodepressants and open the gate to secondary infection.

### Conclusion

From the above mentioned results it can be concluded that the fish infested with internal parasites as nematodes had the highest effect on liver and kidney functions in the studied fish. Preventive measures intended for minimizing the internal parasites which are of significant concern in fish farming and production.

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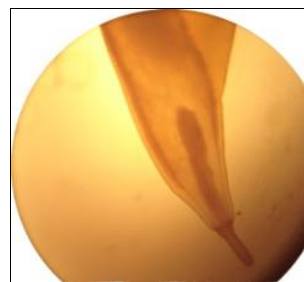
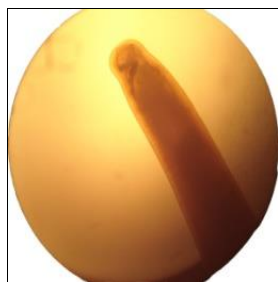
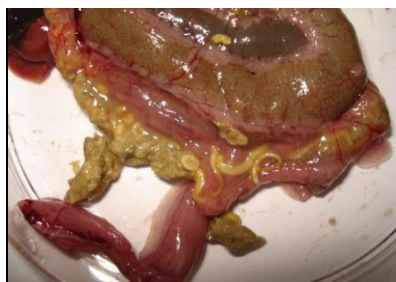
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**Fig. 1:** Congestion with peticeal hemorrhage showing paleness with areas of hemorrhage



**Fig. 2:** Liver of *Siganus revulatus* affecting liver of *Siganus revulatus*



**Fig. 3:** Intestine of *Siganus revulatus* *Nematode.spp* showing heavy infestation of *Nematode.spp revulatus*

**Figs. 4, 5:** Anterior and posterior ends of *Siganus*



**Fig. 6** Paleness of liver in *Lethrinus harak* Excessive mucus secretion *Gerras oyena*

**Figs.7, 8** Anterior and posterior ends of nematode affecting *Lethrinus harak*

**Fig. 11**



**Figs. 9, 10** Anterior and posterior ends of nematode affecting *Lethrinus harak*

**Figs. 12, 13:** Anterior and posterior ends of nematode affecting of *Gerras oyena*





**Figs. 14, 15, 16, 17:** Anterior and posterior ends of nematodes affecting *Gerras oyena*

**Table (1):** Mean ( $\pm$ S.E) Hematological parameters on infested and non-infested Fish with internal parasites in Safaga at Red Sea (n=10):-

Fish species Parameter	<i>Siganus revulatus</i> (N=10)		<i>lethrinus harak</i> (N=10)		<i>Gerras oyena</i> (N=10)	
	Non. infested	infested	Non. infested	infested	Non. infested	infested
RBC (x 10 <sup>6</sup> /μL)	3.17 ±0.10	1.41 ±0.07**	2.64 ± 0.13	1.81 ± 0.12**	2.815 ± 0.75	2.487 ± 0.40
Haemoglobin (g/dL)	11.18 ±0.41	7.93 ±0.39**	15.45 ± 0.52	12.11 ± 0.46**	9.8 ± 2.1	7.9 ± 1.4
Haematocrit (%)	24.88 ±0.91	17.82 ±0.83*	28.1 ± 1.62	22.11 ± 0.95*	41.3 ± 6.8	34.9 ± 2.6
MCV (fL)	113.41 ±4.70	126.66 ±5.78	148.92 ±6.42	132.6 ±5.88	160.8 ± 19.7	129.5 ± 16.2
MCHC (g/dL)	38.44 ±1.59	44.46 ±2.18	24.92 ±2.26	31.81 ±3.52	25.2 ± 6.0	33.1 ± 4.2
WBC (x10 <sup>3</sup> μL)	15.64 ±0.39	18.37 ±0.45*	15.44 ± 0.37	17.69 ±0.41*	14.86 ±1.093	16.56 ±1.28*
Lymphocytes (μL)	12.23 ±0.14	11.72 ±0.12	12.26 ± 0.14	11.92 ± 0.12*	11.19 ±0.13	10.88 ±0.57
Neutrophils (μL)	2.36 ±0.08	4.66 ±0.11*	2.26 ± 0.09	4.98 ± 0.11*	2.69 ±0.09	4.59 ±0.33
Monocytes (μL)	0.15 ±0.07	0.11 ±0.08	0.13 ± 0.05	0.12 ± 0.02	0.13 ±0.02	0.19 ±0.04
Eosinophils (μL)	0.69 ±0.15	1.79 ±0.18	0.63 ± 0.16	1.69 ± 0.19*	0.98 ±0.06	1.22 ±0.16

\*=significant (P<0.05); \*\*= significant (P<0.01)

**Table (2): Mean liver, kidney function tests and plasma glucose of non-infested and infested Fish with internal parasites in Safaga at Red Sea(n=10) :-**

Fish species Parameter	<i>Siganus revulatus</i> ( N=10)		<i>lethrinus harak</i> ( N=10)		<i>Gerras oyena</i> ( N=10)	
	Non. infested	infested	Non. infested	infested	Non. infested	infested
ALT (U/L)	38.74 ±1.72	81.65 ±3.61*	23.33 ±2.34	29.14 ±1.99*	55.29 ±2.26	76.41 ±3.15*
AST (U/L)	54.80 ±2.18	95.1 ±4.74**	32.66 ± 3.16	38.14 ±5.03*	81.66 ±3.25	97.3 ±4.01*
Total protein (g/dL)	4.45 ±0.21	2.81 ±0.19**	3.9 ± 0.26	2.95 ± 0.38*	4.91 ±0.38	3.05 ±0.12*
Urea (mg/dl)	27.54 ±1.58	24.25 ±1.57	36.3 ±1.86	31.5 ±2.06*	41.1 ±1.91	34.35 ±1.52
Creatinene (mg/dl)	0.94 ±0.01	1.10 ±0.02*	1.30 ±0.05	1.61 ±0.01	1.18 ±0.02	1.41 ±0.04
Glucose (mg/dL)	59.4 ±1.35	68.4 ±2.19*	91.24 ±7.31	115.30 ±5.51*	89.9 ± 24.7	122.0 ±20.3

\*=significant (P<0.05); \*\*= significant (P<0.01)

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