



## Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946  
Journal homepage: <https://ejah.journals.ekb.eg/>

### Effect of isopoda on the health status of gilthead seabream (*Sparus aurata*)

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#### Article History

Received in 27/09/2020  
Received in revised from  
2/11/2020  
Accepted in 15/12/2020

#### Keywords:

Isopoda  
*Sparus aurata*  
*Nerocila* spp  
Malathion

#### ABSTRACT

A total number of 100 fish samples (50 apparently healthy fish and 50 seemed to be unhealthy fish) were randomly collected from one farm of cultured Seabream fish at Domeitta provenance, Egypt. Specimens were subjected to parasitological examination for detection of isopoda infestation. The clinical signs of isopoda infested fish are erosion on gill lamella, damages on gill rakers, pale gills, hemorrhagic areas on gill cover, abdomen and on the bases of fins also abrasions and body emaciation was noticed. The detected parasites were *Nerocila* spp. Juvenile. The parasites detected in the gill chamber of investigated fish. These parasites were described in details. Water samples as well as blood samples were taken for determination of water quality and changes in blood parameters due to isopoda infestation. Analysis of water samples revealed that the parasitic infestations levels have increased with decreasing level of dissolved oxygen, increasing ammonia levels, temperature, hardness, pH and salinity. Parasitic infestation resulted in remarkable decline in RBCs, hemoglobin, monocytes values together with noticeable increase lymphocytes, basophils, eosinophils and neutrophils counts. Further, trials for treatment trials of isopoda have revealed that isopoda was not affected by any concentration of all tested chemicals except the high concentration (0.150 ppm) of malathion after six days of treatment.

#### INTRODUCTION

Marine sector of the aquaculture industry is expected to develop in the next 20 years in Egypt especially with limitation of freshwater resources and decline of the valuable commercial wild capture fisheries. The expansion and

intensification of fish cultures will be challenged by several factors especially diseases. Fish parasites and diseases constitute one of the most important problems facing the fishery biologist (Ravichandran et al. 2009).

Most parasitic crustaceans of marine as well

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DOI: 10.21608/EJAH.2021.133607

as fresh water fish can be seen by the naked eyes as they attach to the gills, body and fins of the fish and it spent a large part of their life on fish, possessing their adhesive organs and mouth parts adapted for piercing and sucking fish blood (El Moghazy 2008).

Isopods considered as a large ectoparasites crustacean group on marine fish, diverse and occur on fish worldwide (Abd El Aal and El Ashram 2011). Isopods infesting fish were expected to increase and numerous of isopod species awaited discovery, especially in the tropical and subtropical regions. According to Bray and Justine (2011) about 4000 Isopod species in terrestrial, marine, brackish and freshwater habitats were identified.

Cymothoid isopod causes serious problems to host fish either directly or indirectly affecting the physiological status of the host (Ravichandran et al. 2009; Dash et al. 2014).

Woo (2006) mentioned that the suborder *Cymothoidae* contains about 500 species that parasitize fish.

According to Trilles (1986) 46 species of *Cymothoidae* were reported in Africa (12 *Anilocrinae* and 34 *Cymothoinae*). They were fed on blood and macerated tissues; several species settled in the buccal cavity of fish, others lived in the gill chambers or on the body surface including the fins (Woo 2006; Ravichandran et al. 2009).

In the Mediterranean, the production of sea bass *Dicentrarchus labrax* and sea bream *Sparus auratus* has increased rapidly in the last decade (Eissa et al. 2014).

Rapid increases such as these are linked with the appearance of a number of new host-parasite associations, which may result from hosts being reared in new geographic areas or from indigenous hosts being reared in different environmental conditions (Eman and Engy 2013).

In Egypt culturist collect feral fingerlings marine fishes to be cultured. The isopods have been transferred from the feral fish to the farmed species due to the increasing populations of the latter (Fricke et al. 2011).

Abd El-Aal and El-Ashram (2011) reported that, the incidence of parasitic marine isopods among wild 100 *Argyrops filamentosus* fish was studied. The detected parasite was

*Cymothoa spinipalpa* which observed in the gill chamber and buccal cavity of the host. Slight protrusion of gill cover (operculum), atrophy and hemorrhage at site of attachment were noticed.

Economic losses due to ectoparasite infestation not only result from direct harm to fish, but also from disfigurement which renders fish grown for food and ornamental fish unsuitable for sale, thus impose a big loss to fish industry (Miller et al. 2010).

Little is known about the marine isopods in Egypt expected by Hassan (2001), Eissa (2002), Ali and Aboesa (2007), Abd el all and El Ashram (2011) and Eman et al. (2014) because the species concepts are weakly established in the literatures. Therefore, the present investigations were conducted to view a light on isopoda among sea bream fish from Mediterranean Sea in Damietta province.

Eiras (1994) stated that Copepods are the most numerous among parasitic crustaceans and may be the most common group of fish parasites. They have been found parasitizing skin, gills, eyes, fins and even inside the mouth of fishes, near the palate and nostrils Damage caused by fish parasites includes hemorrhagic and ulcerated lesions, with potential for secondary infection. Some additional effects may be: anemia, retarded growth, loss of weight and loss of equilibrium.

## MATERIALS AND METHODS

A total number of 100 marine fish samples *Sparus aurata* (50 apparently healthy fish and 50 appeared unhealthy fish) were randomly collected in summer season from different sites in farm of cultured marine fish at Domietta provenance, the collected fish measured nearly 20:25cm in length and 200:300gm in weight then transported immediately to the laboratory of Kafr El-sheikh branch of Animal Health Research Institute alive in thick polyethylene bags and tanks containing 1/3 of its volume the water from the site of capture where the remaining volume filled with air.

**Clinical picture:** Clinical examination was made externally and internally on the live or freshly dead fish for detection of any clinical abnormalities according to Conroy and Her-

man (1981).

**Parasitological examination:** fish were examined macroscopically and microscopically for detecting parasites the identification of isolated isopods was identified according to **Bruca (1978)**.

**Preparation of detected isopods for identification:** the detected isopods collected and then kept in a small tube, washed and cleaned by distilled water they fixed in 3% formalin saline and preserved in equal amount of 70% ethyl alcohol-5% glycerine in test tube and permanent amounts prepared by passage in descending grades of alcohol, cleared in glycerin and mounted in glycerin-gelatin, according to **Lucky (1977)**.

**Preparation of water sample for chemical examinations:** water sample from the different pond of collected fish prepared for measuring the chemical parameters dissolved oxygen, nitrite, sulphate, PH., ammonia, salinity, and hardness. The water physico-chemical properties measured were: dissolved oxygen (D.O) (measured by a dissolved oxygen meter), percent of water salinity (measured by a Salinometer), pH values (measured by a pH meter), and unionized ammonia and sulphate measured by special kits.

**Preparation of blood sample for differential leucocytic counts and immune parameters measurement.**

Fresh blood samples were collected from the caudal vessels using disposable plastic syringes according to **Noga (1996)**. The blood samples were then divided into 2 clean, small, dry glass tubes; apart of the blood sample was taken into a glass tube and left for coagulation at room temperature for 5 minutes, refrigerated for 2 hours and then centrifuged at 300rpm for 5-10 minutes. The clear sera were aspirated and kept at -20c, according to **Stoskopf (1993)** and until use for serum biochemical analysis. The other part of blood sample was placed into another glass tube with anticoagulant (EDTA), 4-5 ml blood according to **Noga (1996)** for hematological examination.

For differential leucocytic counts and immune parameters measurement, blood films were prepared and stained according to the protocol by **Lucky (1977)** the smears from scrapings were made and left to air dry, then smears were fixed in absolute methanol,

stained by Giemsa stain that freshly prepared and diluted with distilled water before staining with (10 ml of the stain were added to 90 ml of distilled water). The fixed films were dipped into the prepared diluted stain for twenty minutes. After staining, the films were rinsed with running tap water and air dried. The stained preparations were examined with an oil immersion objective lens.

#### **6-Treatment trials at the farm by using different chemicals.**

- a- Using Formalin 2 ml/m<sup>3</sup> and 3 ml/m<sup>3</sup> respectively for 7 days.
- b- Using Copper sulphate 2 gm/m<sup>3</sup> and 3 gm/m<sup>3</sup>, respectively for 7 days.
- c- Using Malathion 0.125 ppm and 0.150 ppm respectively for 7 days.

At the laboratory the fish subjected to clinical, postmortem and parasitological examinations according to **Amlacher (1970)** Isopods were removed from the host fish; their location and its density were noted. Also, prevalence among the examined fish was calculated. Isopod specimens were collected from the gill chambers and buccal cavity and immediately preserved in 70% ethanol.

#### **Statistical analysis**

The statistical analysis was made using t-test for comparison between seemed healthy group and Isopoda-infested group for testing the significance of the different group in different variables under the study that facilitated the incidences of Isopoda (water quality and immune parameters). The statistical analysis was done according to **(SAS 2004)**.

## **RESULTS**

### **1-Clinical examination of naturally infested fishes:**

The clinical picture in the naturally infested fishes with isopodes were opened mouth and degeneration of the gill filaments, presence of juvenile's parasite in the gill chamber or buccal cavity of different infested fish (Fig., 3). Slight protrusion of gill cover (operculum), and when removed isopods from the site of attachment we found atrophy and paleness at site of attachment (Fig., 4). and retardation of growth in comparison with the non-infested fish in the same age were observed (Fig., 1 & 2 ).

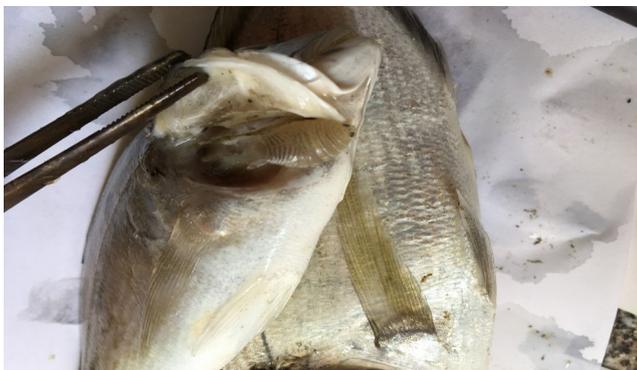


Figure (1) Gross macroscopic picture showed slight protrusion of gill cover (operculum) plus emaciation, due to isopoda infestation



Figure (2) Atrophy and hemorrhage at site of attachment and retardation of growth.



Figure (3) Opened mouth and degeneration of gill filament due to juvenile isopoda embedded in gill chamber.



Figure (4) Pale and atrophy of gills when remove the juvenile parasites

## 2-The morphological description of the parasites.

Kingdom: *Animalia*

Subphylum: *Crustacea*

Family: *Cymothoidae*

Phylum: *Arthropoda*

Order: *Isopoda*

Genus: *Nerocila*

*Nerocila Juveniles* (eagathoid stage) were isolated from gills of examined sea bream fish. In general, the juveniles of most species of *Cymothoidae* resemble slender version of adult males. The primary differences between the adult male and juveniles is the larger eyes of the later and the distinct differences in uropod and pleonite morphology. Eyes very large in proportional to the head, covering about one third the dorsal surface

of cephalon. Medial and distal articles of antennae setose. Uropoda different in shape from adults and highly setose in margins. Exopoda with terminal spine. Distal margin of pleotelson highly setose. Body very slender, length 10 mm and width of pereonite five 3.1mm. (Fig. 5 and 6).

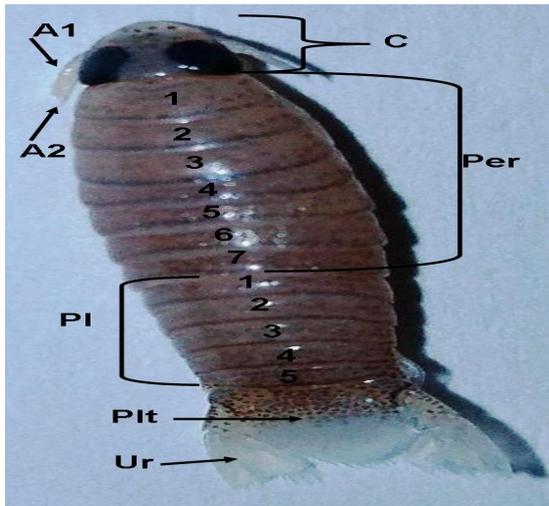


Fig (5) Dorsal view of Juvenile *Nerocila* spp: A1=Antenna 1; A2= Antenna 2; C= Cephalon; Per = Pereon; Pl= Pleon; Plt= pleotelson; Ur= Uropod . x (40)

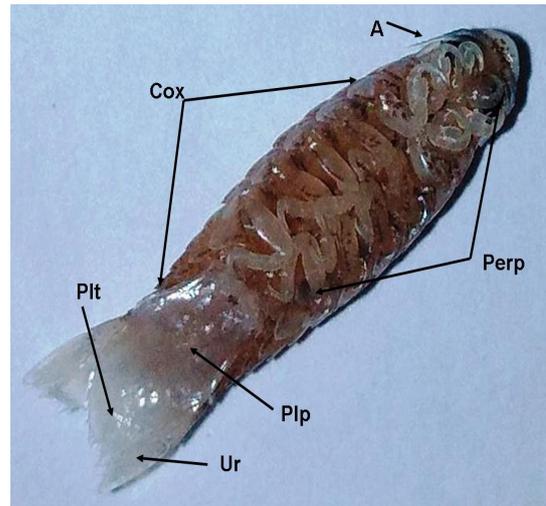


Fig (6) Ventral view of Juvenile *Nerocila* spp: A= Antennae; Perp= Pereopods; Cox= Coxal plates; Plp= Pleopodes; Plt= pleotelson; Ur= Uropod . x(40).

The parasite was found in between the operculum on one side of the infested sea bream and weak tissue damage was noticed on the host fish (PlateC:.1 and 2). The mouthparts are often styliform. The head is not embedded in first segment of the peraeon. Pleon (abdomen) is markedly narrower and shorter than peraeon and consists of six segments; each of the first five segments carries a pair of bi-ramous natory limbs (pleopods). The sixth segment is called the pleotelson, which is flanked by the bi-ramous uropods. Both appeared without marginal setation. Uropods with exopods titled so as not to be fully seen in dorsal aspect,

slight to deep notch often present on medial margin. The pereon, the largest part of the body is composed of the cephalothorax, where the head is unsegmented and bears two pairs of antennae as well as two large black eyes. It consists of seven segments; each carries a pair of appendages (peraeopods). Such legs bearing segments that are clearly separate from each other and end with spine. Based on the morphological characters, these crustaceans are related to ***Cymothoidae, Nerocila* spp.**

### 3-Factors affecting infestation of Isopoda: - a-Water quality

Table (1) Relationships between water quality parameters and Isopoda infestations in Seabream fish.

| Type of fish          | No. of samples | Water quality parameters |               |               |             |                           |                  |                 |                |
|-----------------------|----------------|--------------------------|---------------|---------------|-------------|---------------------------|------------------|-----------------|----------------|
|                       |                | Dissolved oxygen (ppm)   | Nitrite (PPT) | Sulfate (PPT) | PH          | Un ionized ammonia (mg/L) | Temperature (°C) | Hardness (mg/L) | Salinity (ppt) |
| Isopoda infested fish | 25             | 5.4±0.14B                | 0.06±0.001A   | 564±6.41A     | 7.04± 1.14B | 3.0±0.13A                 | 27±2.17A         | 173.6±3.17A     | 19 ±0.17A      |
| Non infested fish     | 25             | 5.8±0.15A                | 0.03± 0.001B  | 500±10.5B     | 7.21±0.22A  | 2.0±0.12B                 | 27.8±2.77A       | 167.5±2.15B     | 18±0.18B       |

Means within the same column of different litters are significantly different at ( $P < 0.05$ ).

### b-Effect of Isopoda on blood and differential leucocytic counts: -

Table (2) Blood and differential leucocytic counts associated with isopoda infestation in seabream.

| Type of fish          | Differential leucocytic counts ( D.L.C ) |            |             |              |           |             |             |             |
|-----------------------|--|------------|-------------|--------------|-----------|-------------|-------------|-------------|
|                       | RBC $10^8$ mm                            | Hb (g/dl)  | Hct(%)      | Lymphocyte % | Monocyte  | Eosinophil  | Basophils   | Neutrophils |
| Isopoda infested fish | 2.26±0.07B                               | 7.63±0.09B | 25.38±1.10B | 59±4.55A     | 1.5±0.22A | 28.30±2.12A | 15.66±1.17A | 10.66±1.16A |
| Non infested fish     | 3.15±0.09A                               | 8.96±0.36A | 42.50±1.36A | 46.66±4.35B  | 2±0.22A   | 22±2.18B    | 10±1.18B    | 9±1.12B     |

Means within the same column of different litters are significantly different at ( $P < 0.01$ ).

### c- Biochemical and immune parameters changes due to Isopoda infestation:

Table (3) Biochemical and some immune parameters changes associated with parasites infestation in seabream fish.

| Type of fish          | Biochemical parameters |              |            |
|-----------------------|------------------------|--------------|------------|
|                       | Cortisol               | Glucose      | Lysozymes  |
| Isopoda infested fish | 1.19±0.01A             | 222.51±5.22B | 0.35±0.0A  |
| Non infested fish     | 0.47±0.02B             | 267.66±7.66A | 0.22±0.02B |

Means within the same column of different litters are significantly different at ( $P < 0.01$ ).

**d- Oxidative biomarkers changes associated with parasitism in cultured seabream:**

Table (4) Oxidative biomarkers changes associated with parasitism in cultured seabream

| Type of fish          | Oxidative biomarkers |                  |           |
|-----------------------|----------------------|------------------|-----------|
|                       | Glutathione          | Super peroxidase | Catalase  |
| Isopoda infested fish | 0.41±0.02B           | 0.066±0.02A      | 2.5±0.15A |
| Non infested fish     | 0.59±0.05A           | 0.031±0.01B      | 2.3±0.13B |

Means within the same column of different litters are significantly different at (P < 0.01).

**e- Treatment by using different chemicals.**

by using formalin, copper sulphate and malathion applied daily for 7 days

Table (5) results of the effect of chemicals on the isopoda infested the cultured seabream inside the farm:

| Formalin           | 1 <sup>st</sup> day | 2 <sup>nd</sup> day | 3 <sup>rd</sup> day | 4 <sup>th</sup> day | 5 <sup>th</sup> day | 6 <sup>th</sup> day | 7 <sup>th</sup> day |
|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| 2ml/m <sup>3</sup> | +                   | +                   | +                   | +                   | +                   | +                   | +                   |
| 3ml/m <sup>3</sup> | +                   | +                   | +                   | +                   | +                   | +                   | +                   |
| Copper sulphat     | 1 <sup>st</sup> day | 2 <sup>nd</sup> day | 3 <sup>rd</sup> day | 4 <sup>th</sup> day | 5 <sup>th</sup> day | 6 <sup>th</sup> day | 7 <sup>th</sup> day |
| 2gm/m <sup>3</sup> | +                   | +                   | +                   | +                   | +                   | +                   | +                   |
| 3gm/m <sup>3</sup> | +                   | +                   | +                   | +                   | +                   | +                   | +                   |
| Malathion          | 1 <sup>st</sup> day | 2 <sup>nd</sup> day | 3 <sup>rd</sup> day | 4 <sup>th</sup> day | 5 <sup>th</sup> day | 6 <sup>th</sup> day | 7 <sup>th</sup> day |
| 0.125ppm           | +                   | +                   | +                   | +                   | +                   | +                   | +                   |
| 0.150ppm           | +                   | +                   | +                   | +                   | -                   | -                   | -                   |

- parasites still active

+ parasites loss activity and stop move

The data illustrated in tables (5) revealed that the isopoda not affected by any of concentration of all tested chemicals at any concentration at the all different periods except the high concentration 0.150ppm after 5days in case of malathion but in case of formalin and copper sulphate not have any effect on isopoda.

**DISCUSSION**

*Cymothoid* isopoda are parasitic crustaceans typically marine and usually inhabit the warmer seas, they are blood feeding, settle in the gill chamber or in the body surface cause serious effects on the fish population and lead to fish mortality and finally high significant economic losses **Eissa et al. (2012)**.

The main clinical signs showed in naturally infested seabream with crustacean infestations were respiratory distress, surface swimming,

bulging of opercula, sluggish movement, and emaciation. In addition, hemorrhagic areas on gill cover. Our results may be attributed to the low respired oxygen of destructed gill epithelium which caused by feeding activity, attachment, fixation and locomotion of isopods causing massive destruction of respiratory epithelial cells. These results are in agreement with those reported by **Eissa et al. (2012)**. The main clinical signs in naturally infested fish with *Nerocila* were respiratory distress, surface swimming, bulging of opercula, sluggish movement and hemorrhages of gills. These results may be endorsed to the reduced rate of respiration due to deteriorated gill epithelium initiated by the feeding activity, attachment, fixation, and movement of the crustaceans. Also, the emaciation recorded in fish infested with isopods may have been a result of a re-

duced appetite for food (**Nagasawa 2004**) Crustaceans reduce growth rates, this result agreed with that recorded by **Costello (2009)**. Regarding the postmortem examination showed that marbling appearance of gills. These result may be attributed to destruction of the different vessels may happen by isopoda, where the blood pressure is low and no extensive haemorrhages were caused and the very short clotting time of blood brings about rapid occlusions of the vessel then thrombus is formed resulting in ischemia, which in turn leads to necrosis. This result agreed with that recorded by **Noor El-Deen (2007)**. These result may be attributed to its attached to the gill filaments using antennae and third legs leading to pathological effects such as erosion, desquamation of tissue, necrosis in branchial epithelial tissue and the severe irritation caused by movement, feeding activity and their claws fixation of such crustaceans which result in asphyxia and then death. These results were similar to that recorded by **Tosken et al. (2008)**.

The results of isopoda infestations among sea bream fish showed that, the higher incidence of isopoda in Seabream attributed to the species differences that causes differences in parasitic infestations among fishes and the larger size of seabream than the size of cultured marine fish. This results agreed with those of **Yatabe et al. (2011)**. Where they reported that, the incidences of fish parasites differ from fish species to another according to the fish immunity, size of fish and degree of infestation with the parasites. Our results agreed with those of **Vainikka et al. (2005)** where they reported that, the level of immune-stimulants and phagocytic cells decreased in the musculature of higher body weight fish than the small body weight fish that is facilitated the infection with fish parasitic infestation.

The morphological features of *Nerocila juveniles* (eagathoid stage) were coincided with that described by **Brusca (1978)** in northern Gulf of California.

The incidence of isopoda infestation in relation to water quality in seabream isopoda infested fish cleared that, the dissolved oxygen decreased when the parasitic infestations level increased, increasing of unionized ammonia

level, increasing temperature, increasing level of hardness, pH and increasing the salinity level.

This attributed to this conditions considered as the stress factors that causes the increasing secretion of cortisol that causes decrease the fish immunity with facilitation of parasitic infestation among seabream.

Many authors reported that the fish parasites are temperature dependent and it increases with temperature (**Abd El-Aal 2011**). The explanation for the high infection rate with *Glugea anomala* in the examined Grouper fish at high water temperature could be attributed to the excess infected zoo plankton eaten by the susceptible fish. Also, our study reported that pH 7.04 which agree with **Claude (2005)** which reported that the decrease of pH (5-8) can act as a barrier against skin and gill parasitic isopoda infection. This could also explain the high prevalence of *Glugea anomala* infection in Grouper fish in summer (pH 6.6-7.3) and the lower prevalence in winter (pH 5.9-6.8).

The result of the effect of isopoda infestation on blood and differential leucocytic count cleared that significant decrease observed in the number of RBCs, Hb, Hct in isopoda infested fish than non-infested and this agree with **Okamura (2003)** and in our study the decline in Hb value of parasitized fish can be attributed to the weak mobilization of Hb from the palate to the hematopoietic organs and this agree with **Scott and Rogers (1981)**

The results of the effect of isopoda parasitic infestation on biochemical and immune assay cleared that, the cortisol and the lysozymes level showed higher level in isopoda infested fish. This results attributed to the fish Isopoda considered as a stress factors on fish that causes increasing the level of cortisol that associated with decreasing level of serum glucose, while, the level of lysozyme increased to overcome the stress conditions associated with parasitic infections. Our results agreed with **Fent (2007)** where reported that, parasites as well as pollutants also affect the endocrine system of host. A number of substances alter levels of different hormone groups such as sex hormones (endocrine disrupters) or stress hor-

mones (adrenalin, cortisol or corticosterone).

The results of the effect of Isopoda infestation on oxidative biomarkers in sea bream cleared that, the glutathione level ranged from the higher level in non-infested fish by isopoda than the infested one. While, the data cleared that, the super peroxidase level ranged from lower level in control and the higher level that observed in isopoda infested fish and the catalase level ranged from the lower level in non-infested fish and the higher level observed in isopoda infested one.

Our results attributed to parasitic infection induces oxidative stress and a higher level of membrane damage in fish organs due to an imbalance between pro-oxidants and non enzymatic anti oxidants and lead to exacerbate lipid per oxidation which used as a biomarker of pathological effects caused by parasitism and stressful ecological factors (Eissa et al. 2014). Our results also, agreed with those of Eissa et al. (2014) where they reported that, the enzyme activities (Superoxide dismutase, Catalase, Glutathione peroxidase, Glutathione reductase,) in gills, liver and musculature of the parasitism infested fish can be used as an oxidative biomarker.

Our results concluded that, the parasitic infestations level increased with increasing level of oxygen demand, increasing of ammonia level, increasing temperature, increasing level of hardness, pH and increasing the salinity level. Also, the parasitic infestations cause increases the level of lymphocyte and neutrophils. also Isopoda not affected by any concentration of all tested chemicals except the high concentration (0.150 ppm) of malathion after six days of treatment. So the study recommended periodically monitoring of water quality parameters in fish aquaculture and prevent pollution beside minimize the prevalence of parasites.

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