Some Investigations observed in Culture Seabass (*Dicentrarchus labrax* L.) infested with *Lernanthropus kroyeri* and *Nerocila orbignyi* and Exposed to Pollution during different seasons at Dammaitte province

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Abstract: This study was conducted to investigate the effect of crustaceans 'copopoda and isopoda on cultured marine seabass (*Dicentrarchus labrax* L.) to cadmium pollution. A total of 400 adult infested fish collected seasonally from Dammaitte province were clinically examined for isolation and identification of infested parasites. Results obtained revealed some clinical pictures as bulging of opercula, sluggish movement, emaciation and sever erosion and hemorrhages of gills and mortality. The detected parasites were identified as copopoda (*Lernanthropus kroyeri*) and isopoda (*Nerocila orbignyi*).The morphological characteristics of *Lernanthropus kroyeri* van Beneden, 1851 and *Nerocila orbignyi* Guerin-Maneville, 1832 were studied by light microscopy. The total prevalence was (16%) and the summer displayed the highest seasonal prevalence. The relation between fish body weights, lengths and infestation rate were studied. Besides, the relation between heavy metal pollution and parasitic infestation was discussed. The present study was concluded that, there were inversely proportion relationship between cadmium concentration pollution in aquaculture and the prevalence of *Lernanthropus kroyeri* and *Nerocila orbignyi* infestation during in European seabass summer and spring seasons while infestation was disappeared during autumn and winter seasons.
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1. Introduction

European seabass fish products have reached to 19,027 tons in Egypt **(GAFRD, 2011).** The decrease of this production of fish resulted in serious parasitic diseases in Egypt where intensive aquaculture is practices **(Noor El Deen, *et al*., 2012)**. Thus, focus has been placed on parasitic fish diseases in these enterprises and their economic and ecological impact. Parasitic infestations represent the majority of the known infectious diseases affecting fish (**Noga, 2010).** In recent years, crustacean parasitic diseases are becoming more frequent in the aquacultures and considered the more parasitic problem on cultured marine fish **(Tansel and Fatih 2012).** The major groups of Crustaceans fish parasites; are Isopoda and Copepoda **(Öktener and Sezgin, 2000).** *Lernanthropus kroyeri* van Beneden, 1851 has been recorded from Suez Canal area in seabass *Morone labrax* **(Eissa *et al*., 2012).** Also, *Nerocila*is a large genus of Cymothoidae. *Nerocila orbignyi* (Guerin-Maneville, 1832) that attaches to the gills of infested *Dicentrarchus labrax* **(Mladineo, 2003).** Commonly distribution areas of *Nerocila orbignyi* are Mediterranean, Northwest Africa, Red Sea, Egypt **(Trilles, 1994).**The more prevalence parasites infested cultured seabass were *Lernanthropus kroyeri* (Copepoda) and *Nerocila orbignyi* (Isopoda) in marine seabass **(Alas *et al*., 2008 and Eissa *et al*.,2012).** Many fish species are infested by isopods which are found in various parts of fish body, including gillcavity and cause gill damage **(Toksen, 2007).** They provide portals of entry for other pathogens in fish **(Horton and Okamura, 2001).** *Lernanthropus* is the most common genus of parasitic copepods affect ingonly *Dicentrarchus labrax*, which inhabit in warmer waters. Some species of *Lernanthropus* are strictly specific, but many are parasitic on several species of fish belonging to one genus, or on several genera of one family **(Sharp *et al*., 2003).**

Pollutants may affectan intermediate or alternate hosts in parasite life cycleand on free-living life cycle stages of parasite invasion **(Sindermann, 1990).** Pollution stress can influence the prevalence of parasites directly or indirectly by impairing the host's immune response, or the parasite infestation may decrease thehost resistance to toxic pollutants **(Khan and Thulin 1991).** Cadmium is potentially harmful to most organisms even in very low concentrations **(Kaoud and El-Dahshan, 2010)** and present in aquatic environment and gills increased due to domestic and industrial mining **(El Safy *et al*., 2011).** Fish accumulate cadmium to concentration many times higher than present inwater **(Yilmaz, 2005 and Noor El-Deen*et al*., 2011).** The relationship between parasitism and pollution is not simple and in essence involves adouble edged phenomenon in which parasitism may increase host susceptibility to toxic pollutants or pollutants may result in an increase or decrease in the prevalence of certain parasites **(Kuperman,1992).**

The present study was directed towards further understanding of marine seabass fish in Ezbat Alborg marine fish farms in Dammaitte region. The objectives were decided to throw the light on the clinical picture, total & seasonal prevalence of the crustacean parasitic diseases affecting *Dicentrarchus labrax*. Besides, the infestation rates in relation to body weights & lengths in Dammaitte province. Moreover, the effect of cadmium pollution on fish parasitism in different seasons.

2. Materials and Methods

Fish for examination:

During the year 2011-2012, a total of 400 specimens of European sea bass, *Dicentrarchus labrax*, were collected from cultured in marine fish farm in Azbat El Borg area, (Damietta Province in corresponding to Mediterranean Sea.). Their body weights and lengths were ranged from less than 60 up to 500 gm and 18 to 40 cm, respectively. The fish were obtained seasonally (each 50 fish) from a private fish farms, then transported to the laboratory alive in fiberglass tank containing 2/3 of its volume the same farm and supply with aerator to oxygen.

Water and tissue samples for heavy metal measurements:

Water and fish samples were collected during the year 2011-2012. Eight water samples were collected in different times from the same area , kept in refrigerator and transferred cold to the laboratory for analysis according to APHA (1992). Parasitized fish specimens were dissected freshly to obtain gills, then frozen until ready for acid digestion using Conc, H2SO4 according to the method outlined by EOSQC (1993). Each 0.5gm of collected gill fish samples were well digested using Conc, H2sO4 according to the method outlined by **Cottenie (1980).** The levels of Cd and were determined at central lab of |National Research Center using atomic absorption spectrophotometer (Model Thermo, AA spectrometer, S series, type s4).

Clinical pictures:

The collected European seabass were examined clinically according to the methods described by **Noga (2010)** paying an attention to the sea bass behaviors in the earthen ponds. Fish specimens under investigation were grossly examined for determination of any clinical abnormalities and any external parasite on all fish according to **Woo (2006).**

Parasitological examination:

1. Macroscopic examination:

Macroscopic examination was done for detection of any abnormalities in different parts of fish body by nacked eyes and hand lens. Skin, fins, gills, eyes and opercula were dissected and examined for presence of parasitic crustaceans.

2. Microscopic examinations:

The microscopic parasites were collected by a fine brush, special needle or eye dropper, washed for several times in fresh water until the specimens had died and left in refrigerator at 4̊C to completely relaxed. The crustaceans then fixed in 70% alcohol glycerin, passed through ascending grades of alcohol (70,80,90,95% and absolute) cleared in xylol, mounted in Canada balsam or by clearing in lactophenol and mounted in glycerin gelatin **(Lucky, 1977).** Crustacean parasites were identified according to **Kabata (1979), Badawy (1994) and Al-Zubaidy& Mhaisen(2013).**

Statistical analysis:

Data were analyzed by analysis of variance using the SAS program. **Duncan's multiple-range test (1955)** was used to verify significance of the mean differences among treatments.

3. Results

Clinical examination:

The main clinical signs of naturally infested European seabass showed in the form of respiratory distress, surface swimming, bulging of opercula, sluggish movement, and emaciation and sever erosion and hemorrhages of gills and grayish dotes with inverted V shape egg saces was obvious even macroscopically and Isopoda was found in between the operculum and pectoral fin on one side and weak tissue damage was noticed on the host fish **(Plate A(1and 2).**

Parasitological examination:

A crustacean parasite was collected from gills of European seabass, *Dicentrarchus labrax*. The male body is slender in shape. The mandible has 7 denticles. The first maxilla consists of 3 segments; the terminal is conical, and the basal segment has 2 distal broad spines. The terminal segment of second maxilla is provided of 2 rows of blunt teeth and blunt spines on the inner margins. The third segment has a single distal spine. The exopod has 5 short distal spines and the caudal rami are short. While the endopod has slender bristled seta. The female is somewhat cylindrical and width at the middle of the body. The head separated by a constriction from the rest of the body. The first thoracic leg is biramous, the exopod of the first segment bear blunt distal spines, while, the endopod bear an elongated distal spine. A tiny papilla-like process is located at the base of the endopod. The egg strings are elongated and uniseriate, strongly flattened eggs **(PlateB:1-8).** Based on the morphological characters, such crustaceans are belonged to Lernanthropidae, *Lernanthropus kroyeri*.

Another crustacean parasite was attached to base of gill arch and operculum of European seabass, *Dicentrarchus labrax*. The parasite was found in between the operculum on one side of the infested seabass and weak tissue damage was noticed on the host fish **(PlateC:.1 and 2).** It was dorsoventerally flattened crustaceans with symmetrical body and measured up to 2.4 mm. 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 natatory 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 peraeon, 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). These can be prehensile or ambulatory. Such legs bearing segments that are clearly separate from each other.Based on the morphological characters, these crustaceans are related to Cymothoidae, *Nerocila orbignyi*.

Prevalence of crustacean infestation in seabass fish:

Table (1) shows total and seasonal prevalence of crustacean parasites in examined sea bass fish. A table (2& 3) shows the prevalence in relation to body weights and lengths.

As shown in **table (1)** there is a significant difference ($P < 0.05$) among the total and seasonal prevalence of crustacean parasites in examined sea bass fish. As the total prevalence of infestation was 16% and seasonal prevalence of *Lernanthropus kroyeri* in examined sea bass fish in spring and summer season were 4 and 6% respectively. While, the seasonal prevalence of *Nerocila orbignyi* in examined sea bass fish showed significant differences ($P < 0.05$) in spring and summer season as its prevalence were 2 and 4% respectively. **Tables (2&3)** show the significant differences ($P < 0.05$) of parasitic prevalence in relation to body weights and its lengths were 4 and 6% at body length 20-25 and 25-30 cm and body weight 100-140 and 150-210 gm of seabass respectively.

Heavy metal residues and seasonal variations:

Tables (4) show the residues and concentration of cadmium in gills of examined fish samples in different seasons. Contamination levels of heavy metal in gills were 8.73, 8.77, 5.75 and 4.71 and water 0.0028,0.0031, 0.0030 and 0.0027 in autumn, winter, spring and summer seasons respectively.

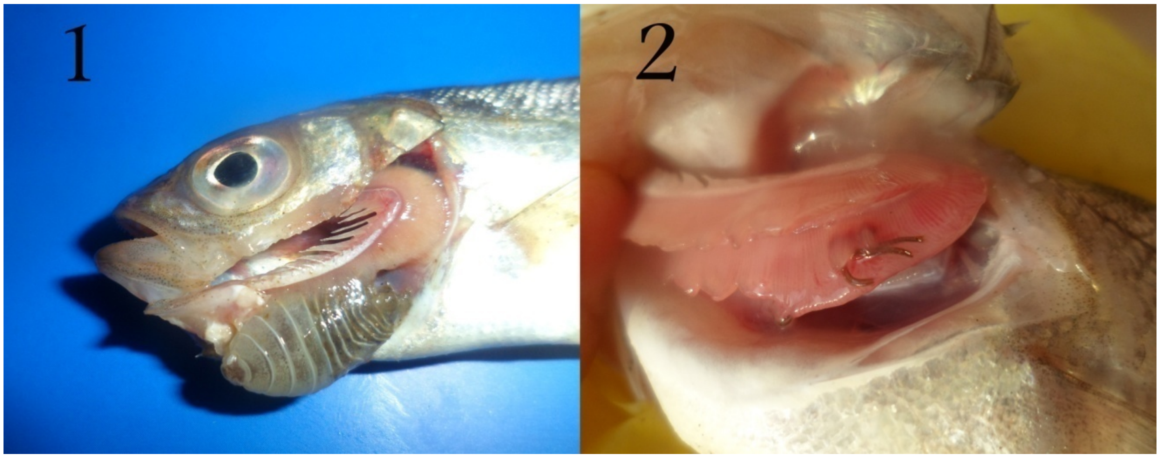

Plate (A): Showing infested seabass with isopoda embedded in gill filament and arch (1) .seabass emaciated seabass infested with heavy infested with copopoda(2) .

Plate (B): Light photomicrograph of female *Lernanthropuskroyeri*(lateral view,1- Venteral view,2 and (anterior part,3, posterior part,4. Light photomicrograph of male (lateral view,5- Venteral view,6 and (anterior part,7, posterior part,8.

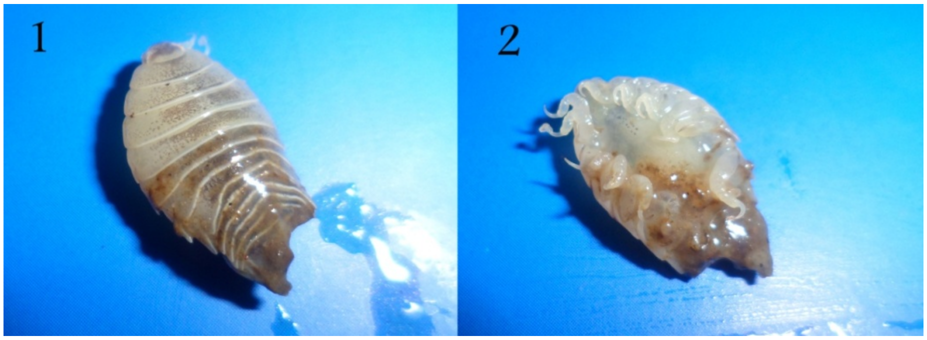

Plate (C): Light photomicrograph of *Nerocila orbignyi* (Dorsal view, 1 and Venteralview, 2

Table, 1.Showing prevalence and intensity of Crustacean parasites in seabass *(Dicentrarchuslabrax*).

Parasite species	Prevalence (%)*				Intensity**		
	Season	No.of examined fish	No.of infested fish	%	Season	Average S.D	Sn-1
Lernanthropuskroyeri	Spring	50	8	4	Spring	A 5.42±5.6	5.82
	Summer	50	12	6	Summer	A 4.58±4.3	4.6
	Autumn	50	0	0	Autumn	C 0	0
	Winter	50	0	0	Winter	C 0	0
Nerocilaorbignyi	Spring	50	4	2	Spring	B 1.55±1.55	1.50
	Summer	50	8	4	Summer	A 4.45±3 .49	4.51
	Autumn	50	0	0	Autumn	C 0	0
	Winter	50	0	0	Winter	C 0	0

Chi^2 = $^{(14.56)**}$; ** = Significant at (P < 0.01)
**Means within the same column of different letters are significantly different at (P < 0.05).

Table (2): Seasonal prevalence of crustacean infestations among seabass

Body length (cm)	No. examined	Crustacean infestation			
		Lernanthropuskroyeri		*Nerocilaorbignyi*	
		No. infected	%	No. infected	%
15- 20	50	0	0	0	0
20- 25	50	8	4	4	2
25- 30	50	12	6	8	4
30- 35	50	0	0	0	0
Total	200	20	10	12	6

Chi2 = (6.88)**; ** = Significant at (P < 0.01) ;

Table (3): Seasonal prevalence of crustacean infestations among seabass

Body Weight (gm)	No. examined	Crustacean infestation			
		Lernanthropuskroyeri		*Nerocilaorbignyi*	
		No. infected	%	No. infected	%
60- 100	50	0	0	0	0
100- 140	50	8	4	4	2
150- 210	50	12	6	8	4
220- 350	50	0	0	0	0
Total	200	20	10	12	6

Chi2 = (7.99)**; ** = Significant at (P < 0.01)

Table (4): Mean heavy metals concentration (ppm) in gills of infested fish in different seasons.

Heavy metal	Permissable limit (mg/Kg)	Seasonal variation in gills (mg/Kg)			
		Au	Wi	Sp	Su
Cadmium in gills	0.05* 0.1**	8.73	8.77	5.75	4.71
Cadmium in water	0.01***	0.0028	0.0031	0.0030	0.0027

Au=Autumn, Wi=Winter, Sp=Spring & Su= Summer.
*FAO/WHO (1992); **EOSQC (1993) & FAO/WHO (1984) and ***Permissible limit according to USEPA (1986)

4. Discussion:

The main clinical signs showed in naturally infested European seabass *Dicentrarchus labrax* with crustacean infestations were respiratory distress, surface swimming, bulging of opercula, sluggish movement, and emaciation and sever erosion and hemorrhages of gills.. In addition, hemorrhagic areas on gill cover and in late stages, external ulcers located in the gill filaments. These results may be attributed to the low respired oxygen of destructed gill epithelium which caused by feeding activity, attachment, fixation and locomotion of crustaceans causing massive destruction of respiratory epithelial cells. These results are in agreement with those reported by **Eissa *et al*. (2012).** Emaciation was recorded in infested seabass may be due to crustacean infestation which reduce fish appetite and became off food, this agreed with **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 efferent vessels may happen by copepod crustaceans and 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 toits 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*., 2006 and Tosken *et al*., 2008.**

The second parasite *Nerocila orbignyi* under discussion isolated from gills and feed on gill tissues of their hosts, which can lead to many problems especially emaciation for small fish. These results may be attributed to the adult isopods are haematophagus (feed on blood) and cause anaemia. in addition to the stationary parasites attaching especially on gills and can seriously reduce the respiratory surface by causing atrophy of the gills on which they press. This result was similar to that recorded by **Horton and Okamura (2001).**The crustacean parasites were isolated from gills of *Dicentrarchus labrax*. This result agree with **Manera and Dezfuli (2003); Mladineo (2003) and Tosken *et al.* (2008)** that isolated the same genus from the same host and site and disagree with**Kayış and Ceylan(2011)** who obtained the same species from gills of *Solea solea*..

The measurements and morphological characters of *Lernanthropus kroyeri*, was nearly similar to that obtained by **Ravichandran *et al*., (2009).** While, *Nerocila orbignyi* was nearly similar to that obtained by **Özak, (2006**).

Concerning the total prevalence of crustacean infestation of *Lernanthropus kroyeri* was 10%. This result is lower than that obtained by **Eissa *et al*., (2012)** who recorded the prevalence within the Red sea seabass (*Dicentrarchus labrax*) was being 47 %. This difference may be attributed to the type of breeding of seabass that wild in Red sea and locality from which fish samples obtained. While, the total prevalence of isopoda was 6%. This result is lower than that obtained by **Horton and Okamura (2001)** who recorded the prevalence within the Turkish sea bass (Dicentrarchus labrax) farm was being 66 %. This difference may be attributed to the age of seabass and locality from which fish samples obtained.

Concerning the seasonal prevalence of crustacean infestation, the peak was highest in summer 10%, followed by spring 6% andabsent in autumn and winter. This disagrees with results obtained by **Eissa *et al*., (2012)** in which he recorded the summer season as the season of the highest infestation rate19%,followed by autumn 17%, while spring 7% and the lowest was 4% in winter season. These results were may be attributed to cultured fish less infested than wild fishand the difference of geographical distribution of hosts and parasites.

Regarding heavy metals concentrated in gills of *seabass* was higher in autumn and winter seas than that in spring and summer seasons. These results may be attributed to increase of water flow in summer and spring seasons than that in autumn which leading to solution of polution and salinity . these results may be agree with that recorded with (**Al-Weher, 2008)** who recorded that certain environmental conditions such as salinity could play an important factor in heavy metals accumulation in the living organisms up to toxic concentrations and cause ecological damage.

Gills were of the higher of all tested of cadmium pollution than water surrounds. These results agreement with that of (**Saeed and Abd-El Mageed, 2008)**. This may be due to the metallothioneins proteins which are synthesized in gills tissue when fishes are exposed to cadmium and detoxify them. Moreover, seasonal variation showed higher residual values in winter, spring and autumn than in summer in both tissues and water. These results are in agreement with these of **Saeed (2007) and El Safy*et al*. (2011)** and disagree with that recorded by **Noor El Deen*et al*.(2011)** who recorded that cadmium concentration in gills of examined *Tilapia zilli* was increased gradually from winter season (0.33±0.0004 ppm) and autumn season (0.43±0.004 ppm) to (0.53±0.003ppm) in spring season and (0.63±0.006 ppm) in summer season. These difference of concentrations and seasons may be attributed to difference of area, water and type of fish.

Finally, the relationship between heavy metal and abundance of parasites, environmental change due to pollutants can influence parasitic-host interaction **Khan and Thulin (1991) andKhan(2012)**. In this study, crustacean infestation were found to be negatively related to heavy metal concentarations in different seasons. This may be attributed to the toxic effect of the heavy metals on the crustaceans which may cut its life cycle **(El-Seify *et al*., 2011)** .On the other hand, no infestation was detected in liver or musculature with high accumulated heavy metals in different seasons. These results are in agreement with that of **Lafferty and Kuris (1999)** who recorded that pollutants may kill sensitive free living stages of the parasite or reduced survival of free-living cercariae and miracidia, leading to a lower prevalence of parasitic larvae**.**

The present study was concluded that, there were inversely proportion relationship between cadmium concentration pollution in aquaculture and the prevalence of gill crustacean infestation during spring and summer seasons while infestation was disappeared during autumn and winter seasons. Also, there was a relationship between cadmium residues in *Dicentrarchus labrax* gills and its concentration in the water, the obtained results showed that the cadmium concentration in the gills were higher than that in the water.

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