Studies on Parasitic Crustaceans among Some Cultured Marine Fishes in Ismailia Governorate

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Abstract

The present study was conducted on a total of 200 marine fishes (100 of *Dicentrarchus labrax* and 100 of *D.punctatus*) collected from different private fish farms in Ismailia Governorate. There were no pathognomonic lesions in the naturally infested fishes except marbling appearance of gills infested by Isopoda. Skin lesions were found in opercular cavities of fishes infested by Isopoda. The main P.M lesions were hemorrhagic areas on EBS and emaciation. Some fishes showed Sea lice in the buccal cavity with ulcerative lesions in the area of attachment. The highest crustacean infestation rate was recorded in *Dicentrarchus labrax* (75%) followed by *D.punctatus* (42%). The isolated crustacean parasites from both fish species were copepods (*Lernanthropus kroyeri*, *Caligus minimus* and *C.longipedis*) and Isopods (Nerocila spp., Renocila spp. and Anilocra spp.).Total and Seasonal prevalence of crustacean parasites, histopathological picture and identification of copepod parasites using molecular biology (PCR) were also recorded.

Introduction

Nobody can deny that food is an essential means of survival for any living organism including human beings. Fish occupy the first place at the top of the human food list, as they are considered a major source of proteins, particularly marine fish which contain large amounts of polyunsaturated fatty acids (PUFAs) that play a role in alleviating the cholesterol level in blood, provide protection from arteriosclerosis, several heart diseases and colon cancer ***(Van Bilsen, 2014)***. Fish also contain minerals and trace elements which are essential for having a healthy life, body anabolism and maintaining the vitality of all animal cells ***(Sivakumar et al., 2007).*** Unluckily, like other vertebrates, fish could be affected by different species of parasites which can even cause major losses, notably to fish offspring under certain conditions. Fish parasites are considered a direct cause of diseases that deteriorate the general health condition of the former and in turn, fish become susceptible to various diseases of other etiology. It is well known that both internal and

external parasitic diseases pose hazardous economical losses in pisciculture. A country like Egypt for instance, is famous for having long periods of optimum warm weather which enhances the ability of external parasites to reproduce more ***(Eissa, 2002)***. The undeniable fact that crustacea and fish exist in the aquatic environments, both marine and freshwater, has helped to throw them into ecological propinquity as fish represent the perfect hosts for any potential parasite.So, it is not surprising for crustaceans which adopt parasitism as their mode of life to be found parasitic on or in fish (external or internal parasites). External parasitic crustaceans are known to have numerous species and abundant individuals. They also have extensive structural diversification and varied effects on their hosts. It is also worth mentioning that those sneaky parasites hold an academic interest, and play an essential economic role as they are capable of inducing mortality of their hosts under specific conditions ***(Dias et al., 2015).***

The major economic losses in fisheries are mainly caused by crustacean diseases which may result in killing, stunting or damaging fish. They may also kill juvenile fishes or impair them which threaten their survival ***(Khamees et al., 2015).*** The present study is directed towards further studies on parasitic crustaceans among some cultured marine fishes notably with the forthcoming projects which are being held along the new Suez Canal in Egypt as aquaculture has an important role in the development and meeting the increase demand for aquatic animal production as it is known that the rapid increase in aquaculture facilities is mainly due to the decline in ocean fisheries worldwide ***(Rosenberg, 2008)***.

The objectives of the present study were decided to throw the spotlight on:

1-Collection of available cultured marine fishes.

2-Recording the clinical picture (signs and lesions).

3-Isolation and identification of the causative agents by traditional and advanced techniques.

4-Recording the total and seasonal prevalence of the crustacean diseases in the examined fishes.

5-Recording the histopathological alterations induced by such diseases.

Materials & Methods

Fishes:

A total number of 200 live or freshly dead fishes (100 *Dicentrarchus labrax* and 100 *D.punctatus*) of different lengths and various body weights were randomly collected from different private fish farms in Ismailia Governorate. The collected live fishes were kept in polyethylene bags containing 1/3 of their volume water, while the remaining volume

was filled with air.

Clinical picture:

First, body weight and total length of the examined fishes were recorded, then clinical examination was done on the two fish species (live or freshly dead ones). Fish specimens were grossly examined for detection of any crustacean parasites and any clinical abnormalities. The postmortem examination was performed on all fishes according to ***Almacker (1970)***.

Parasitological examination:

- **Macroscopic examination:**

Macroscopic examination was done by the naked eyes and magnifying lens to detect any abnormalities on the external body surface of fish. Skin, eyes, gills, fins, opercula and mouth cavity were dissected and examined for the presence of any crustacean parasites.

- **Microscopic examination:**

Freshly sacrificed fishes were scraped from just behind the operculum to the tip of the tail fin with a scalpel blade. Mucus and scales were transferred to slides with a drop of distilled water and cover slips were placed over them to prevent drying and then, examined microscopically **(Lucky,1977)**.

- **Smear preparations, permanent slides:**

The attached crustaceans were collected, recovered and detached by a dissecting needle and a fine brush, kept in small vials and washed several times with distilled water, fixed in 3% formalin and preserved in equal amounts of 70% alcohol-5% glycerin in test tubes. Permanent slides were prepared by passing them in ascending grades of glycerin alcohol (30,50,70,90 and 100%), cleared in glycerin and mounted in glycerin-gelatin **(Lucky,1977)**, then examined microscopically.

- **DNA extraction and amplification:**

- Sample was washed one time with SDS 1%.
- 180 µl of ATL buffer was added to the larvae and 20 µl QIAGEN protease into the bottom of a 1.5 ml microcentrifuge tube. The tube was incubated at 56˚C till tissue lysis.

C- 200 µl buffer AL were added to the sample, mixed by pulse vortexing for 15 seconds. The mixture was Incubated at 72˚C for 10 min.

D- The mixture was Incubated at 56˚C for 10 min.

E- 1.5 ml microcentrifuge tube were centrifugated to remove drops from the inside of the lid.

F- 200 µl ethanol (96%) were added to the sample, and mixed again by pulse vortexing for 15 seconds. After mixing, the 1.5 ml microcentrifuge tube was briefly centrifugated to remove drops from the inside of the lid.

G- The mixture from step 6 was carefully applied to the QIAamp mini spin column (in a 2ml collecting tube) without wetting the rim. the cap was closed, and centrifugated at 8000 rpm for 1 min.

The QIAamp mini spin column was placed in a clean 2 ml collection tube, and the tube containing the filtrate was discarded.

H- The QIAamp mini spin column was carefully opened and 500 ml buffer AW1 were added without wetting the rim. The cap was closed, and centrifugated at 8000 rpm for 1 min. The QIAamp mini spin column was placed in a clean 2 ml collection tube, and the tube containing the filtrate was discarded.

I- The QIAamp mini spin column was carefully opened and 500 ml buffer AW2 were added without wetting the rim. The cap was closed, and centrifugated at full speed for 3 min.

J- The QIAamp mini spin column was placed in a new 2 ml collection tube and the old collection tube was discarded with the filtrate. Centrifugation at full speed for 1 min was done.

K- The QIAamp mini spin column was placed in a clean 1.5 ml microcentrifuge tube, and the collection tube containing the filtrate was discarded. The QIAamp mini spin column was carefully opened and 100 µl buffer AE were added. The QIAamp mini spin column was incubated at room temperature (15-25˚C) for 1 min, and then centrifugated at 8000 rpm for 1 min.

L- DNA was amplified by using specific primers 28S rRNA having the sequence (TGA ACA GGG TAA AGC CCA TCA C) and (GGA TGG TGT AAA CGA AAG ATG).

Histopathological examination:

Tissue specimens from the infested organs (gills and skin) were taken, fixed immediately in 10% neutral buffered formalin, dehydrated, blocked in paraffin wax, sectioned at 5-7 microns and stained with H & E stain according to ***Carleton (1976)***.

Results:

Clinical picture:

The clinical examination of the naturally infested fishes (*Dicentrarchus labrax* and *D. punctatus*) showed no obvious clinical abnormalities except in the case of heavy infestation with the copepod crustacean parasites Caligus spp. and Lernanthropus spp. The clinical signs were emaciation and hemorrhagic areas on external body surface, distress, excessive mucous secretion, sluggishness and sometimes the fishes rubbed their bodies against hard objects. Opercular bulging can also be seen in the presence of isopoda which may even lead to a complete sloughing of gill filaments in one or two gill arches. An opercular skin lesion can be seen after the removal of isopoda from the gill chamber of the infested fish which resulted from the attachment of the former to the latter (Plate 1).

Results of parasitological examination:

1-Crustaceans isolated from *Dicentrarchus labrax* and *D. punctatus*:

I-Copepods:

A)*Lernanthropus kroyeri* Van Beneden,1851. A crustacean copepod isolated from the gills of both *D.labrax* and *D.punctatus*. The bodies of both male and female isolated copepods appeared elongate in both sexes. The cephalothorax of the female has a dorsal shield narrower anteriorly,posterior margin slightly concave, rounded posterolateral corners, anterolateral extended ventrally as prominent, rounded lobes. A deep constriction is found between the cephalothorax and pregenital trunk. The female is easily recognized by the presence of the two egg-sacs which were clearly seen macroscopically in the gills of the examined fish specimens. Total length is up to 3-4 mm (Plates 2&3).

B) *Caligus minimus* Otto,1821

A crustacean parasite isolated from the buccal cavity of both *Dicentrarchus labrax* and *D. punctatus*. It is commonly known as sea louse. It has 2 pairs of antennae with 2 lunules at each side of the cephalothorax and 4 legs. The thorax is segmented to 4 leg-bearing.The male is characterized only by the first and second antennae of the parasites. The genital segment is slightly oblong and not broadly enlarged as that in the female. The female is characterized by the presence of rounded mature and immature eggs found in 2 egg-sacs. Total length is about 3-5mm (Plate 4).

C) *Caligus longipedis*

This crustacean parasite was also isolated from the buccal cavities of *Dicentrarchus labrax and D.punctatus* . It's also another member of the sea lice parasites. It has moderately separated lunules. The cephalosome is more than ½ of the total body length. The genital complex is wider than long and much longer than the abdomen. The caudal rami are as long as the abdomen. Microscopically, it differs from all other members of the genus by having crescent-shaped sclerotized areas on the last segment of the inside branch of leg 2. Maximum female length is 3.8-5.5mm, while that of male is 2.4-5.5mm (Plate 5).

II- Isopods:

3 different types of isopoda belonging to 3 genera were found in the branchial cavities of the examined specimens of *Dicentrarchus labrax* and *D.punctatus*. The parasites were attached to the anterior-ventral portion of the branchial chambers. one parasite was usually found in one side of the gill chambers and not at both sides and sometimes the parasite is found attached to the external body surface (Plate 6). The 3 types of isopoda are Nerocila spp.(Plate 7) , Renocila spp.(Plate 8) and Anilocra spp. (Plate 9).

Nerocila sp. Leach, 1818

Isolated from *D.labrax* and *D.punctatus*

Large sessile eyes well developed

Body weakly twisted to one side

and slight vaulted.
Cephalon width greater than length and narrows anterior to rounded apex.
Cephalon weakly immersed in center of pereonite 1
Narrowly antenna reaching about midline of pereonite1, antennule reaching posterior margin of pereonite 2.
Pereonite one has no anteolateral angles extension to cephlon.
Antennule shorter than antenna.and its bases widely separated.
Coxal plates prominent reaching to posterior margin of their respective pereonites
Pleonites subequal in length, widest at pereonite 4 or 5;
Pereonites 6 and 7 posterolateral margins not produced.
Pereopods from1 to7 without spine.
Pleon not immersed in pereonite 7.
Uropodal rami wide and subequal to posterior margin of pleotelson.

Renocila sp. Trilles, 1962

Isolated from *D.labrax* and *D.punctatus*
Large sessile eyes well developed
Body narrow, Pleonites one width as long as 2, 3,4pleonites.
pereonite 5 & 6 same width, pereonite 7 smaller than 5&6, dorsal surface smooth.
Coxal plate 5-7 shape prominent
Dorsal surface with scattered chromatophores, concentrated on posterior borders of segments.
Cephalon with rostrum folded back, posterior margin indented
Cephalon not immersed in pereonite.
Antenna bases widely separated and expanded subequal to antennule.
Antennule longer and broader than antenna
Pereopods from1 to7 without spine.
Exopod rami longer than endopod.
Uropodal rami wide and longer than posterior margin of pleotelson

Anilocra sp. Leach,1818

Isolated from *D.labrax* and *D.punctatus*
Large sessile eyes well developed
Body wide nearly oval , Widest at pereonite 4
Cephalon truncate appearance in dorsal aspect
Cephalon weakly immersed in pereonite 1
Coxal plates small compact not reaching level of posterior margin of respective pereonites
Pereopods increasing in length posteriorly, pereopod 7 longer than 6.
Narrowly antenna reaching about midline of pereonite1, antennule reaching posterior margin of pereonite 2.
Pereonite one has small anteolateral angles extension to cephlon not reach to eyes.
Antenna bases widely separated and expanded subequal to antennule.
Pleon slightly immersed in pereonite 7.
Pereopods from1 to7 without spine.
Uropodal rami wide and longer than posterior margin of pleotelson.

2- Identification of family Caligidae by using PCR:

The target gene (Caligus spp.) was identified by using 28S rRNA

specific primers having the sequence (TGA ACA GGG TAA AGC CCA TCA C) and (GGA TGG TGT AAA CGA AAG ATG). PCR amplification and agarose gel electrophoresis yielded a positive result of the used sample (*Caligus minimus* and *C.longipedis*) of 575 bp (Photo 1).

3- Prevalence of crustacean parasites infestation among the examined fishes:

Tables (1,2&3) and their charts show the total and seasonal prevalence of crustacean infestations among the examined *D.labrax* and *D.punctatus*

Tables (4,5&6) and their charts show the total prevalence of infestation with *Lernanthropus kroyeri* , Caligus spp. and Isopoda respectively in the examined fishes.

Table (7) and its chart shows prevalence of crustacean infestations in relation to fish sex.

6-Histopathological examination of the infested fishes:

A-The skin and musculature of both *Dicentrarchus labrax* and *D.punctatus* infested with isopoda revealed vacuolar and ballooning degeneration in the epidermal cells with focal superficial sloughing to deep ulceration. The dermis exhibited edema and focal mononuclear cell infiltration. The underlying muscles showed massive hyaline degeneration and Zenker's necrosis with focal mononuclear cells infiltration and melanophage cells (Plate10)

B-The gills of *Dicentrarchus labrax* infested with isopoda revealed marked destruction in the primary and secondary gill lamellae. The gill arch exhibited edema and numerous mononuclear cell infiltration. The muscular tissue was edematous and degenerated with mononuclear cell infiltration (Plate 11).

C-The gills of *Dicentrarchus punctatus* infested with isopoda showed focal desquamation in the secondary lamellae with mononuclear cells infiltrated the primary and secondary lamellae (Plate 11).

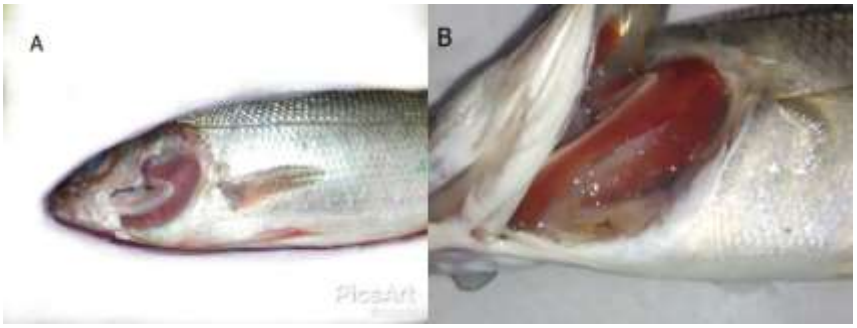

Plate (1): *Dicentrarchus labrax* showing excessive mucus secretion in gills *D. labrax* showing skin lesion and sloughing of gill filaments after the removal of isopoda

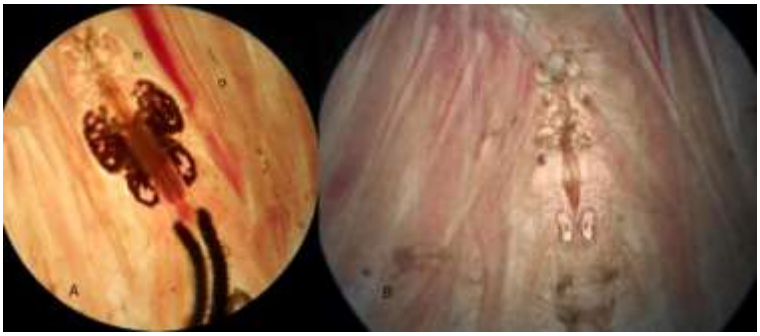

Plate (2) : A. Female *Lernanthropus kroyeri*
B.Male *L. kroyeri* both isolated from the gills of *Dicentrarchus labrax* and *D. punctatus*

Plate (3): A. Cephalothorax of *Lernanathropus kroyeri* , A1: first antenna; A2: second antenna; Mt: mouth tube; M2: second maxilla; M: maxilliped; L1: first thoracic leg; Ce: cephalothorax; L2= Second leg, L3= Third leg. **B**. **Male *Lernanthropus kroyeri*:** A1= First antenna, A2= Second antenna, Ce= Cephalothorax, L3= Third leg, L4= fourth legs, Up= Uropods, a= abdomen, ss = spermatophore sac. **C. Female of *Lernanathropus kroyeri* :** L3:Third leg; L4: fourth leg; Es: egg sac**;** a = abdomen; Gf = Gill filament

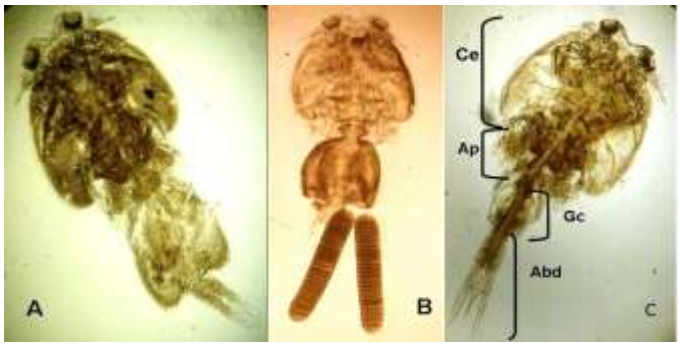

Plate (4): A. Adult female *Caligus minimus* without egg sacs**. B.** Adult female *Caligus minimus* with egg sacs. **C.** Male *Caligus minimus*: Whole parasite**:**Ce = Cephalothorax; Ap = Apron; Genital complex; Abd = Abdomin

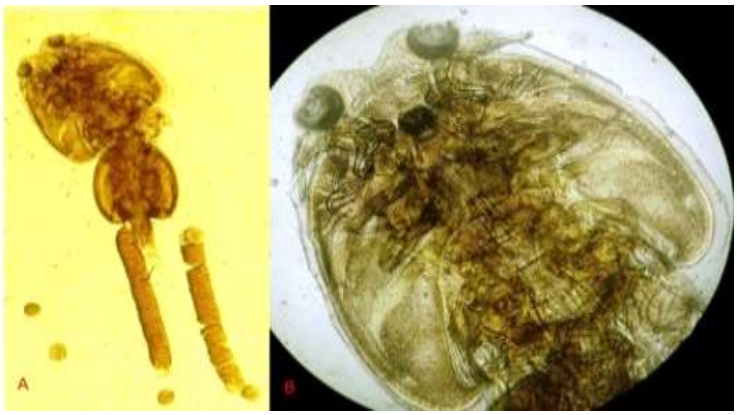

Plate (5):A. Female *Caligus longipedis***. B.** Cephalothorax of *Caligus longipedis*

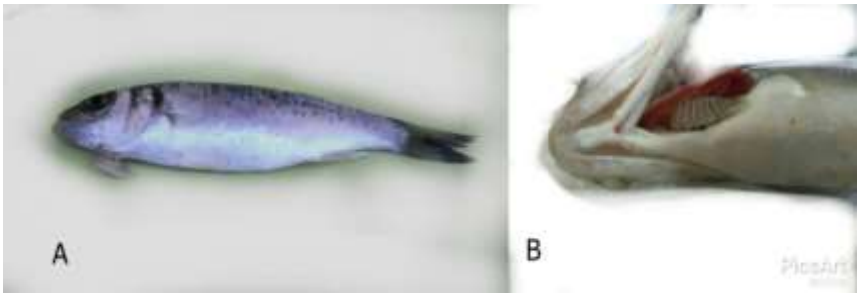

Plate (6): A. An isopoda attached to skin at the base of the head of *Dicentrarchus punctatus.* **B**. An isopoda in the branchial cavity of *Dicentrarchus labrax.*

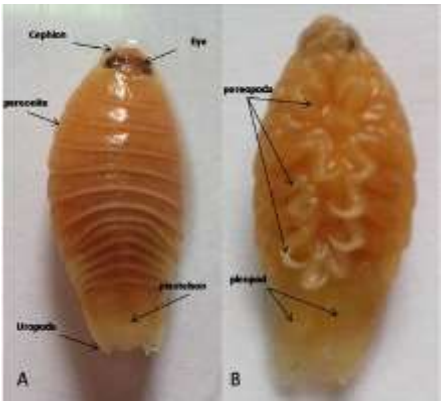

Plate (7): Nerocila sp.
A-Dorsal view **B-** Ventral view

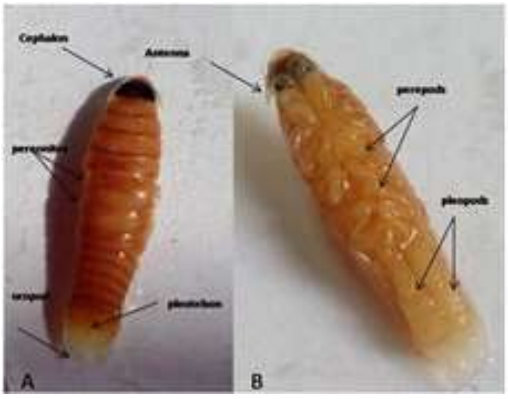

Plate (8): Renocila sp.
A-Dorsal view B- Ventral view

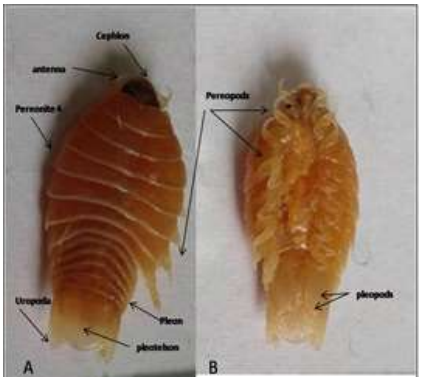

Plate (9): Anilocra sp. A-Dorsal view
B- Ventral view

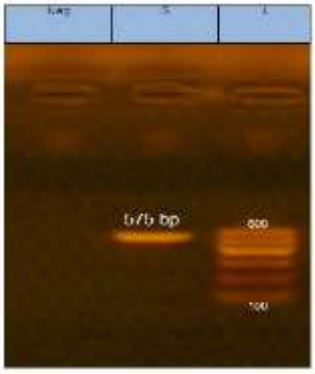

Photo (1): A representative gel displaying analysis of 28S rRNA region from individual adult specimens of Caligus spp. Lane (S) at 575 bp. Lane (L) represents the 100 bp DNA ladder as a marker (bp).

Table (1): Total prevalence of crustacean infestations in examined fish sp.

Fish species	No. of examined fish	No. of infested fish	%
D. labrax	100	75	75
D. punctatus	100	42	42
Total	200	117	58.5

Table (2): Seasonal prevalence of crustacean infestation among the examined *Dicentrarchus labrax*:

Season	Fall (n=25) %	Winter (n=25) %	Spring (n=25) %	Summer (n=25) %	Total (n=100) %
Lernanthropus kroyeri	12	64	84	80	60
Caligus spp.	8	44	68	76	49
Isopoda	4	0	0	12	4

Table (3): Seasonal prevalence of crustacean infestation among the examined *Dicentrarchus punctatus:*

Season	Fall (n=25) %	Winter (n=25) %	Spring (n=25) %	Summer (n=25) %	Total (n=100) %
Lernanthropus kroyeri	32	32	36	32	33
Caligus spp.	0	24	4	0	7
Isopoda	8	0	12	0	5

Table (4): Total prevalence of infestation with *Lernanthropus kroyeri* in examined fish:

Fish species	No. of examined fish	No. of infested fish	%
D. labrax	100	60	60
D. punctatus	100	33	33
Total	200	93	46.5

Table (5): Total prevalence of infestation with Caligus spp. in examined fish:

Fish species	No. of examined fish	No. of infested fish	%
D. labrax	100	49	49
D. punctatus	100	7	7
Total	200	56	28

Table (6): Total prevalence of infestation with Isopoda in examined fish:

Fish species	No. of examined fish	No. of infested fish	%
D. labrax	100	4	4
D. punctatus	100	5	5
Total	200	9	4.5

Table (7): Prevalence of crustacean infestations in relation to fish sex

Fish species	No. of examined fish	No. of infested fish		%	
		Males	Females	Males	Females
D.labrax	100	47	28	47	28
D.punctatus	100	42	23	42	23
Total	200	89	51		

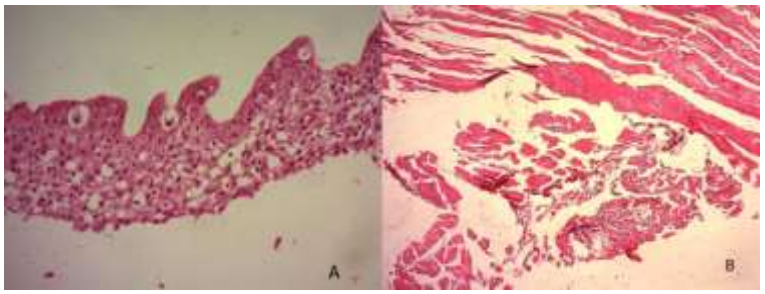

Plate (10): A. Skin of *Dicentrarchus labrax* infested with isopoda, showing advanced vacuolar and ballooning degeneration in the epidermal cells with superficial sloughing. **(H & E stain X 250). B.** Musculature of *Dicentrarchus punctatus* infested with isopoda.The underlying muscles show marked hyaline degeneration and Zenker's necrosis with focal mononuclear cell infiltration and melanomacrophage cells.**(H & E stain X 250)**

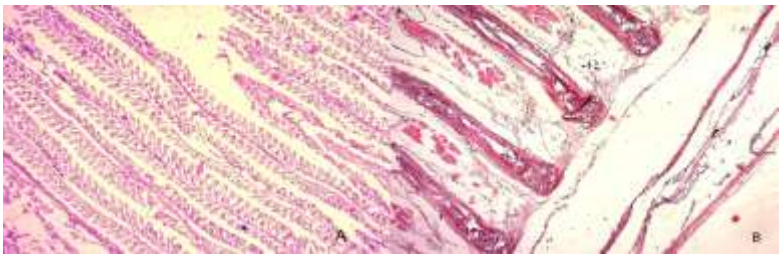

Plate (11): A. Gills of *Dicectrarchus labrax* infested with isopoda showing focal desquamation in the secondary lamellae with mononuclear cell infiltration in the gill lamellae. **(H & E stain X 100). B.** Gills of *Dicentrarchus punctatus* infested with isopoda showing complete sloughing of the secondary lamellae with marked edema in the gill arch and mononuclear cell infiltration in both gill arch and lamellae. **(H & E stain X 250)**

Discussion

The present study deals with some parasitic crustaceans which naturally infest some cultured marine fishes. These fishes are *Dicentrarchus labrax* and *Dicentrarchus punctatus* . They are commonly known as Sea bass and spotted Sea bass, respectively. They were randomly collected from different private fish farms in Ismailia governorate.

The main clinical signs which were observed in the infested fishes, especially those which were heavily infested with copepods were emaciation, distress; excessive mucus secretion, sluggishness and sometimes the fishes rubbed their bodies against hard objects. The previous results are in agreement with ***Toksen (2007)***, ***Eissa et al.(***

2012). Opercular bulging can also be seen in the presence of isopoda which may even lead to a complete sloughing of gill filaments in one or two gill arches accompanied with skin lesions which could be clearly seen in the skin area surrounding the operculum after the removal of isopoda from the opercular chambers. This is in agreement with ***Eissa (2002)*** *and* ***Samah El Shaffey (2016)***.

The parasitological examination revealed two types of crustacean parasites. 3 copepods and 3 isopods. The first parasitic copepod was identified as *Lernanthropus kroyeri* and it was isolated from the gills of both *Dicentrarchus labrax* and *Dicentrarchus punctatus* . This result agrees with ***Eissa et al. (2012)*** *and* ***Engy El- Raziky (2016)*** who isolated the same genus from the same host and the same site, while it differs from that obtained by ***Noor El-Deen et al.(2013)*** *and* ***Dawlat Hassanin (2016)*** who isolated the same parasite from other fish species such as *Mugil cephalus* and *Moolgarda seheli*.

The second copepod under discussion was identified as *Caligus minimus* Otto, 1821.

The parasite was isolated from the buccal cavities of both *Dicentrarchus labrax* and *Dicentrarchus punctatus* . This result is in agreement with ***Ragias et al.(2004)*** *and* ***Noor El-Deen et al.(2013)*** who collected the same parasite from the same fish species and the same site, while it counteracts that obtained by ***Toksen (2015)*** who isolated the same parasite from the gills of cultured gilthead sea bream *Sparus aurata* and***(Engy El- Raziky ,2016)*** who collected the same parasite from the gills and inner surface of the operculum of *Dicentrarchus labrax* .

The third copepod under discussion was identified as *Caligus longipedis* and it was also isolated from the buccal cavities of both *Dicentrarchus labrax* and *Dicentrarchus punctatus*. This result is different from that obtained by ***Venmathi et al. (2009)*** who collected the same parasite from the gill cavities of *Gnathonodon speciosus* . It is also different from that obtained by ***Rganeshamurthy et al. (2014)*** who collected the same parasite from the body surface of 2 species of cultured marine ornamental fish larvae which are *Amphiprion percula* and *A.clarkii.*

The first isopod under discussion was identified up to genus only and it is known as Nerocila spp. It was isolated from the branchial cavities of both *Dicentrarchus labrax* and *Dicentrarchus punctatus*. The parasite was found attached to the anterior-ventral portion of the branchial chambers of the examined fish species. It was usually found in one side of the gill chambers and not at both sides. It was also found attached to the skin at the base of the head. This result is in a partial agreement with ***Ali et al. (2008)*** who found the crustacean isopod

Nerocila bivittata on the caudal peduncle(skin) of the Rusty Blenny *Parablennius sanguinolentus*. It also partially agrees with ***Ahmet (2014)*** who isolated *N. bivittata* from the skin of the brown meagre Sciana *umbra.*

The second isopod under discussion was identified as Renocila spp. and it was also isolated from the gill chambers of both *Dicentrarchus labrax* and *Dicentrarchus punctatus*. This result is in agreement with ***Eman et al. (2014)*** who isolated the same genus *Renocila thresherorum* from the sea bass *Morone labrax*, while it counteracts that obtained by ***Williams et al. (2006)*** who isolated Renocila spp .from different marine fishes in Venezuela.

The third and final isopod under discussion was identified as Anilocra spp .and was also isolated from the branchial chambers of both *Dicentrarchus labrax* and *Dicentrarchus punctatus*. This result counteracts that obtained by ***Williams et al. (2006)*** who isolated *Anilocra efhaemuli* from *Heteropriancanthus cruentatus*. It also counteracts that obtained by ***Eman et al. (2014)*** who isolated *Anilocra meridionalis* from Sardinella spp., as they isolated the same parasite from different fish species.

In this study, PCR was used in the identification of family Caligidae by using 28S rRNA specific primers. PCR amplification and agarose gel electrophoresis showed a positive result of the used sample (*Caligus minimus* and *C.longipedis*) at 575 bp. This result is slightly compatible with that obtained by ***Muhd Faizul Hasmi (2013)*** who used the same 28S rRNA primers to perform a phylogenetic study on 3 different Caligus spp. which were *C.chiastos* and *C.epidemicus* as they both yielded fragments consisting with 3096 bp, while *C.rotundigenitalis* yielded 3120 bp respectively. Other few trials have been made concerning the identification of Caligus spp. but by using different types of primers such as ***(Freeman et al.,2013)*** who performed a study of multiple gene analyses of caligid copepods by using SSU rRNA universal primers (390 fwd and 870 rev.) which yielded 1772 bp for *Pseudocaligus fugu* and 1776 bp for *P.uniartus* respectively. The differences in results are definitely attributed to the different types and numbers of Caligus spp. samples used in PCR, and the different primers used in the same study.

The total crustacean infestations in the examined fish species was 58.5%. This result is a lot higher than that obtained by ***Maather EL-Lamie(2007)***, as it was 15.67%, ***Eman El Boghdady(2015*)** which was 41.9%, ***Engy El-Raziky(2009)*** which was 7% and finally, ***Engy El-Raziky(2016)*** which was 18%. This result is closer to that obtained by ***Doaa Faisal (2008)*** which was 60%, but from a different fish species *Mugil cephalus*. This

difference could be due to the difference of locality from which fish samples were collected and the difference of fish species themselves.

The seasonal prevalence of crustacean infestation among *Dicentrarchus labrax* was estimated for each parasite separately. The total seasonal Lernanthropus infestation was 60%. It was the highest in spring 84%, followed by summer 80%, winter 64% and finally Fall 12%. This result is in disagreement with ***Ola Abu Samak and Ashraf (2008)*** who found that the infestation rates with the same parasite reached their maximum values (42.5% and 35%) in fall and winter respectively, while their minimum value was 7.5% in spring.

The seasonal prevalence of Caligus infestation was 49%. It was the highest in summer 76%, followed by spring 68%, winter 44% and Fall 8% respectively. This sequence slightly agrees with ***Eissa et al. (2012)*** who isolated *Lernanthropus pscianae* and *Caligus carangis* from *Morone labrax* and found that the highest infestation was recorded in summer 76% , while winter was the lowest season 16%. This result and sequence disagrees with ***Banu et al. (2014)*** who examined specimens of the European Sea bass *Dicentrarchus labrax* which were infested with *Caligus minimus*. They noticed a gradual increase in the prevalence of such parasite from fall to winter, then reached its maximum value in spring and finally, declined to the minimum in summer.

The total seasonal prevalence of Isopoda infestation was 4%. It was the highest in summer 12%, followed by fall 4%, while it was 0% in both winter and spring. This result is lower than that obtained by ***Gehan Shager and Ahmed (2006)*** who found that the total seasonal prevalence of parasitic isopods in some marine fishes was 10.7%. This result also a bit lower than that obtained by ***Ali et al. (2008)*** who found the crustacean isopod *Nerocila bivittata* on the caudal peduncle of the Rusty Blenny *Parablennius sanguinolentus* with a total seasonal prevalence of 7.4%.

The previous results could be attributed to the differences of the geographical distribution of both hosts and parasites, besides that each parasite has its own optimum environmental temperature at which it reaches its highest infestation peak.

Regarding the seasonal prevalence of crustacean infestation among *Dicentrarchus punctatus*. The total seasonal *Lernanthropus* infestation was 33%. It was the highest in spring 36%, while it gave equal rates in the rest of the seasons (Fall, winter & summer), as it was 32% in each season. This result counteracts that recorded by ***Eman El-Boghdady (2015)*** who noticed a gradual seasonal increase in

Lernanthropus infestation in *Dicentrarchus punctatus*. It was at its lowest value in spring and increased gradually in summer, followed by winter, and reached its highest peak in fall.

The total seasonal prevalence of Caligus infestation was 7%. The highest value was in winter 24%, followed by spring 4%, and it gave equal rates in both summer and Fall 0%. This result is relatively in accordance with that obtained by ***Eman El-Boghdady (2015)*** who noticed that the highest infestation rate of the same parasite was in both Fall and winter, while it was at its lowest value in spring and summer. This result disagrees with that recorded by ***Doaa Faisal (2008)*** who found that the prevalence of Caligus *curtus* among *Mugil cephalus* was 12%. The highest seasonal infestation rate was recorded in spring 20%, then summer 16%, Fall 8% and finally, winter 4%.

The total seasonal prevalence of Isopoda was 5%. It was the highest in spring 12%, followed by Fall 8% and it was equal in both winter and summer 0%. This sequence disagrees with that obtained by ***Eman El-Boghdady (2015)*** who found that the highest seasonal prevalence of Nerocila spp. in *Dicentrarchus punctatus* was in fall, followed by summer and the lowest values were in both winter and spring. This difference could be attributed to the difference of the geographical distribution of both hosts and parasites, and the optimum temperature required for the flourishing of each parasite.

The total prevalence of infestation with *Lernanthropus kroyeri* was 46.5%. This result is closer to that recorded by ***Eissa et al. (2012)*** which was 47%, but it is much higher than that recorded by ***Manera and Dezfuli (2003)*** which was 35%. This difference in results could be attributed to the different localities from which fish samples were collected.

The total prevalence of infestation with sea lice or Caligus spp. was 28%. This result is closer to that obtained by ***Banu et al. (2014)*** who found *Caligus minimus* among examined specimens of *Dicentrarchus labrax* with a prevalence of 29.8%. This result is lower than that recorded by ***Engy El-Raziky (2016)*** which was 36% in the same fish species. This result is much higher and even disagrees with ***Jiann Hsiung et al. (2001)*** who detected Caligus spp. among the grey mullet with a total prevalence of 5.3%. It also disagrees with ***Abd El-Aal (2003)*** who estimated the overall infestation rate of *Caligus elongatus* in 3 marine fish species and it was 10.43%. These differences could also be attributed to the differences of both localities and the collected fish specimens.

The total prevalence of infestation with Isopoda was 4.5%. This result in a closer agreement with ***Costa and Chellappa (2010)*** who

recorded a total prevalence of Isopoda 5.9% in *Chloroscombrus chrysurus*. This result is a bit lower and in accordance with that recorded by ***Eman et al. (2014)*** who isolated *Renocila thresherorum* from *Morone labrax* with a prevalence of 6%, while it disagrees in fish species with the same scientists who isolated *Anilocra meridionalis* from Sardinella spp. with a prevalence of 4%. This result is a bit lower than that obtained by ***Ali et al. (2008)*** who collected the crustacean Isopod *Nerocila bivittata* with a prevalence of 7.4% It is also much lower and disagrees with ***Ahmet (2014)*** *and* ***Samah El-Shafey (2016)***, as the former isolated the Isopod *Nerocila bivittata* from the brown meagre with a total prevalence of 16.98%, while the latter was 33.5% among 3 different marine fish species. I could assume that these differences in results were due to the human interference, as I was informed by fishermen from whom fish specimens were collected that they removed the Isopods from fish once they found them accidently so that the marketability of fish would not be affected, so this low result was not attributed to natural environmental conditions.

The present study revealed that male fishes showed the highest infestation rate with crustacean parasites more than female ones, as the total prevalence of crustacean infestation in the males of *Dicentrarchus labrax* was 47% while that in females was 28%. The same thing was encountered in the second fish species *Dicentrarchus punctatus* , as the total prevalence of crustacean infestation in males was 42% while that in females was 23%. This result agrees with ***Banu et al. (2014)*** who indicated that the infestation with sea lice in the European Sea bass *D.labrax* was not influenced by host's sex, age and size. This result agrees also with that obtained by ***Takele et al. (2016)*** who found that male fishes were highly infested with some crustacean parasites than female ones in some commercial fish species in the Southern Gulf of Lake Tana, Ethiopia.

Excess parasitism in males may not be as a result of immune competence. Quantitative differences in parasite infection between sexes can be expected and may be explained as a consequence of different habitats occupied by males and females, differences in diet and/or physiology.

The gills of the infested fishes revealed marked destruction in the primary and secondary gill lamellae accompanied by focal desquamation in the secondary gill lamellae. The muscular tissue was edematous and degenerated with mononuclear cell infiltration. This result is in agreement with ***Noor El-Deen et al. (2013)*** *and* ***Mohamed et al.(2015)***. The skin and musculature of the infested fishes revealed vacuolar and ballooning degeneration in the epidermal cells with focal

superficial sloughing to deep ulceration and edema. This result is in agreement with ***Kania et al. (2010)*** and ***Noor El-Deen et al.(2013)***.Last and not least, we hope that the people responsible for aquaculture projects would not undermine the dangerous impacts which result from crustacean infestations of fish and put them under serious considerations.

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الملخص العربى

تم تجميع 200 سمكة بحرية (100 من سمكة القاروص و 100 من سمكة القاروص النقط عشوائيا من بعض المزارع السمكية الخاصة فى محافظة الإسماعيلية. لم توجد علامات مرضية مميزة فى الأسماك المصابة طبيعياً عدا الظاهرة الرخامية لخياشيم الأسماك المصابة بالأيزوبودا. بعض التقرحات الجلدية كانت موجودة فى التجويف الخيشومى للأسماك المصابة بمتماثلة الأرجل (الأيزوبودا). أسفر الفحص التشريحى عن وجود أنزفة على السطح الخارجى للجسم مصحوبة بالهزال. بعض الأسماك كانت تحتوى على قمل البحر فى التجويف الفمى و الذى نتج عنه حدوث خدوش و تقرحات. أعلى نسبة إصابة بالقشريات الطفيلية كانت فى سمك القاروص (75%)، يليها القاروص النقط (42%). الطفيليات المعزولة من نوعى السمك كانت الكوبيبودا (ليرنانثروبس كروير، كاليجاس مينيماس و كاليجاس لونجيبيداس) الى جانب ثلاثة أجناس من متماثلة الأرجل (نيروسيلا ، رينوسيلا ، و أنيلوكرا).و قد تم رصد النسبة الموسمية و العلاقة بين اوزان الأسماك و أطوالها و نسبة الإصابة و الصورة الهستوباثولوجية إلى جانب تصنيف طفيل (الكاليجاس) باستخدام الطرق التقليدية و عن طريق تفاعل البلمرة المتسلسل تم رصدهم جميعاً.