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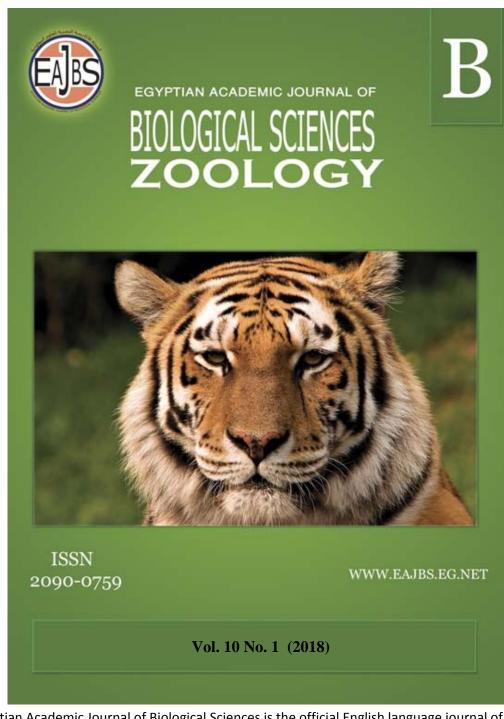
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Infestation Study of *Livoneca redmanii* (Isopoda, Cymothoidae) on *Mugil cephalus* in Lake Qarun, Egypt

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ABSTRACT

The present study deals with the infestation study of *Livoneca redmanii* (Isopoda, Cymothoidae) on *Mugil cephalus* in Lake Qarun, Egypt. Out of 576 examined fish collected monthly from the different localities of Lake Qarun during the period (January - December, 2016) there are 269 (46.7 %) fish were infested by crustacean parasite. Results showed that the highest infestation percentage in *Mugil cephalus* was 76.20 % recorded in February and the lowest one occurred in January (19.40 %). Generally, the infestation percentage in male fishes is lower than those in females being, 34.6 % and 65.4 % respectively. In case of males the highest infestation percentage occurred in July being, 65.5 %, while in case of females the highest percentage was 72.1 % in March.

The isopod parasite, *Livoneca redmanii*, preferred medium size fish (14-17 cm) with infestation percentage 70.64 % and fishes that had weight ranged between 12 g and 14 g with infestation percentage being, 78.82 %. Also, this parasitic isopod infected gill bilateral and/or unilateral infection per hosted fish with percentage being 98.1 %. The main clinical signs in infected fishes appeared in slow swimming at the water surface, extensive mucus secretion, increased opercula movement and some fishes aggregated on the surface and accumulated at the fresh water inlet. Examination of post mortem lesions revealed that presence of ulcer and erosion blew the gills at the site of parasite and skin abnormalities such as skin ulcers, scale-less and discoloration.

Histopathological examination of infected gills showed hyperplasia of epithelial cells in gill filament. Hyperplasia of lamellar epithelium and mucous secreting cells tend to be confusion between secondary gill lamellae, lifting of secondary lamellae and congested blood vessels were also noticed. It is now clear that cymothoid isopod, *Livoneca redmanii* was causes economic loss associated with reduced fish growth, high mortality and marked drop in fish production from Lake Qarun.

INTRODUCTION

Crustacean ectoparasites are more frequently encountered in the aquaculture industry (Tansel and Fatih, 2012). It was found that about 25% of parasites infesting fish considered being crustaceans that are classified into three main categories named copepod, brachiura and isopod (Eiras *et al.*, 2000 & Öktener and Sezgin, 2000). Isopods occur on the outer body surface, fins, inside the mouth, the gills, nostrils, or occasionally burrowing in special tunnels in the musculature of their hosts (Hoffman, 1998). Moreover, they were considered to be the largest ectoparasites that infest fishes worldwide (Rhode, 2005) and cause significant economic losses to fisheries, not only through mortalities, stunting growth or damaging tissues of the fish (Bunkley-Williams *et al.*, 2006; Toksen, 2007), but also by acts as vector for transmission of other fish pathogenic organisms (Horton and Okamura, 2001).

Alas *et al.* (2008) documented that the life cycle of Cymothoidae is considered to be holoxenic cycle that involve only one host (The final host). Additionally, these parasites take place worldwide in various habitats, mainly in the seaside regions (Sullivan and Stimmelmayr, 2008). Cymothoid isopods are permanent ectoparasites of marine and freshwater fishes causing serious problems to host fishes either directly or indirectly (Ravichandran *et al.*, 2009). They survive primarily on a hematophagus diet (blood and macerated tissues) (Woo, 2006), causing anemia and death in small fish (Ravi and Raj kumar, 2007).

Lake Qarun constitutes a very important sector in the Egyptian fisheries, for both significant total catch and a large number of economically important species. *Mugil cephalus, M. Capito, M. saliens, Liza aurata, Solea solea* and *T. zillii* were the dominant fish species in the lake waters. *Dicentrarchus labrax, Sparus aurata, Anguilla anguilla* and *O. niloticus* are rarely found in the commercial catch. The fries of mullets, sea bass and sea bream were mechanical transported from El-Max Station in Mediterranean Sea to this lake (Khalaf-Allah, 2001; Hassan, 2002; Ghanem, 2006 & 2011).

The introducing of fish fry from the Mediterranean Sea and other sources into the lake causes accidental introduction to other non-commercial species in Lake Qarun. Nine of small fish (*Trachyramphus bicoarctatus, Aphanius dispar, Aphanius fasciatus, Aphanius spp. (hybrid), Atherina boyeri, Atherinomorus lacunosus, Pomatoschistus marmoratus, Silhouattea egyptia and Gambusia affinis*) are introduced to Lake Qarun (Zaid, 2006 and Khalaf–Allah, 2014).

Afifi (2015) mentioned that small fish were not collected during November in all sites at Lake Qarun. The disappearance of small fish at Lake Qarun, may be due to the heavily abundance of crustacean parasites, which come from Mediterranean Sea with fish fry and caused severe damage to fish stock in the lake.

The problem in Lake Qarun that prospected in the present study, where of isopod infestation among fishes was noticed causing fish loss and marketing problems. Therefore, the present study aimed to provide information on the prevalence, site of infection, clinical signs and histopathological examination for infestation of *Livoneca redmanii* (Isopoda, Cymothoidae) on *Mugil cephalus* at Lake Qarun, Egypt.

MATERIALS AND METHODS

The study area:

The study area in this work included the South coast of Lake Qarun, Egypt. Lake Qarun is located about 80 Km southwest of Cairo and lies between longitudes of 30°.41778` & 30°.8275` E and latitudes 29°.44194` & 29°.51111` N in the lowest part of El-Fayum depression (Fig., 1).

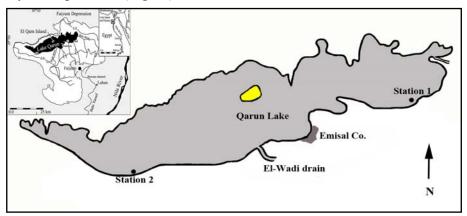


Fig. (1): Map of Lake Qarun showing the study area.

Fish samples collection:

A total of 576 of *Mugil cephalus* specimens were collected monthly from different localities of Lake Qarun, during the period from January to December, 2016. Gill net, encircling net and traps were the main fishing method used to collect the fishes. Wherever possible fishes were examined fresh or preserved in 10% formalin solution for latter examination. In the laboratory, fishes were taxonomically identified according to Bishai and Khalil (1997). Total and standard lengths were measured to the nearest millimeter and recorded. Fishes were also weighted to the nearest 0.1 gram and then the following studies were carried out. Crustacean parasites were collected from the hosted fish and taxonomically identified, as far as possible up to genera according to Brusca (1981).

Site of infection and Clinical examination:

All fishes were totally inspected for any clinical abnormalities and any ectoparasitic infestations according to **Woo** (2006). The infested fishes were carefully examined at the site of isopod infestation to determine its target organ in the host and photographed.

Histopathological examination:

For histopathological examination, normal and infested fish tissues were taken from the parasite attachment area by mouthparts and appendages in skin and gills of species studied, immediately fixed in alcoholic Bouin's solution for at least 24 hours, dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene and embedded in wax (M.P.: 58°C). Vertical sections were cut at 4-6 μ in thickness stained with Harris's haematoxylin and eosin (Humason, 1979) for routine histological examination. Finally, the slides were microscopically examined and photographed by using camera mounted on light microscope and described.

Data analysis:

Statistical analysis and graphs of data was conducted by using Microsoft Excel, 2007 under windows programs.

RESULTS

Prevalence of crustacean parasites among examined *Mugil cephalus*:

Data in Table (1) showed that the total number of infested fishes was 269 (46.7%) from 576 collected fish. Generally, the infested fishes were infected by single or double parasites during all months, except infested fishes during January, July, August and October they were with single infection.

Concerning months, the highest infestation percentage was 76.20 % recorded in February and the lowest one 19.4 % occurred in January. The infestation percentage during the remaining months fluctuated between 21.40 % in August to 63.00 % in May. Notice the absent record of data in December (Table, 1 and Fig.2).

Table (1): Prevalence infestation percentage of the parasite *Livoneca redmanii* on *Mugil cephalus* at Lake Qarun, during the year 2016.

Months	Number of examined	Infested fishes		
wontins	fishes	Number	Percentage	
January	31	6	19.40	
February	63	48	76.20	
March	87	43	49.40	
April	67	26	38.80	
May	46	29	63.00	
June	50	31	62.00	
July	69	29	42.00	
August	56	12	21.40	
September	39	18	46.20	
October	39	16	41.00	
November	29	11	37.90	
December				
Total	576	269		

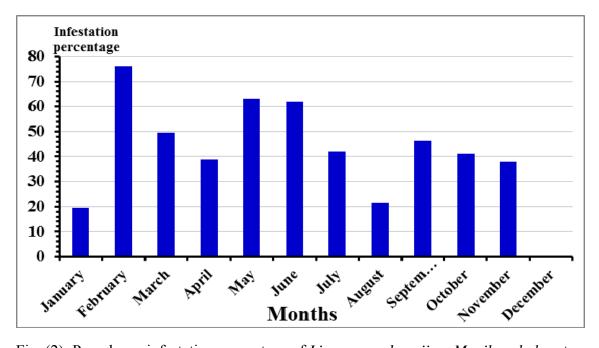


Fig. (2): Prevalence infestation percentage of *Livoneca redmanii* on *Mugil cephalus* at Lake Qarun, during the year 2016.

Prevalence of crustacean parasites in relation to the sex of *Mugil cephalus*:

Results in Table (2) showed that the infestation percentage for male fishes approximately equal half the same percentage for females, being 34.6 % and 65.4 % respectively. Concerning with months, the infestation percentages for males and females of *M. cephalus* fluctuated from month to another. The highest infestation percentage in males recorded during July, being 65.5 % and the lowest one occurred in August (16.7 %). It is entirely absent during January. The infestation percentage in the remaining months fluctuated between 27.3 % in November and 50.0 % in October (Fig. 3). On the other hand, in case of females, the highest infestation percentage recorded during January being, 100 % and the lowest one occurred in July (34.5 %). The infestation percentage in the remaining months fluctuated between 50.0 % in October and 83.3 % in August (Fig. 3).

	Total number of infested fish	Male		Female	
Months		infested fish no.	Percentage	infested fish no	Percentage
January	6			6	100
February	48	14	29.2	34	70.8
March	43	12	27.9	31	72.1
April	26	8	30.8	18	69.2
May	29	8	27.6	21	72.4
June	31	12	38.7	19	61.3
July	29	19	65.5	10	34.5
August	12	2	16.7	10	83.3
September	18	7	38.9	11	61.1
October	16	8	50.0	8	50.0
November	11	3	27.3	8	72.7
December					
Total	269	93	34.6	176	65.4

Table (2): Prevalence infestation percentage of Livoneca redmanii on males
and females of Mugil cephalus at Lake Qarun, during the year 2016.

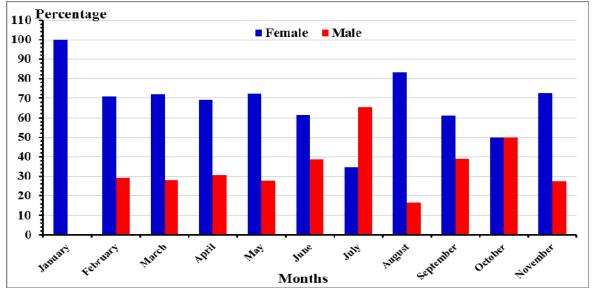


Fig. (3): Prevalence infestation percentage of *Livoneca redmanii* on male and female *Mugil cephalus* at Lake Qarun, during the year 2016.

Prevalence of crustacean parasites in relation to length and weight of *Mugil cephalus*:

Results in Table (3) showed the number of infested fish was 269 (46.7 %) from all examined fishes. The highest infestation percentage was 22.68 % at 15 cm fish length, while the lowest on was 0.37 at 21-22 cm fish length. The other percentages fluctuated between 0.74 % and 10.78 % at 10 cm and 17 cm fish length respectively (Fig. 4). The data showed that the most infested fishes concentrated at medium size fish length (14 – 17 cm) being, 70.64 %. On the other hand, the small and large fish length had low infestation percentages being, 16.72 % and 12.64 % respectively (Table, 3). Also, the results showed that there is no infested fishes had length more than 23 cm.

Data in Table (3) showed that the highest infestation percentage was 33.09 % and 31.23 % recorded at 40 g and 30 g fish weight respectively. While the lowest one was 0.37 % at 90 g fish weight. The remaining percentages varied from 2.97 % at 70 g fish weight to 14.5 % at 50 g fish weight (Fig. 5). There are no infested fish has weight more than 90 g. Also, the data showed that the infested fishes concentrated at weight 12-14 g being, 78.82 %, however, the smallest fish weights (1-11 g) had very low infestation percentage being, 13.1 %.

Table (3): Prevalence infestation percentage of *Livoneca redmanii* on *Mugil cephalus* in relation to length and weight groups at Lake Oarun during the year 2016

Length (cm)			Weight (g)		
Size class (cm)	# of infested fish	%	Weight groups	# of infested fish	%
10	2	0.74	10	1	0.37
11	9	3.35	20	34	12.64
12	14	5.20	30	84	31.23
13	20	7.43	40	89	33.09
14	43	15.99	50	39	14.50
15	61	22.68	60	13	4.83
16	57	21.19	70	8	2.97
17	29	10.78	80		
18	16	5.95	90	1	0.37
19	11	4.09	100		
20	5	1.86	110		
21	1	0.37			
22	1	0.37			

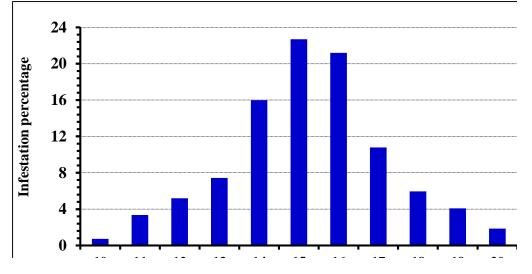


Fig. (4): Prevalence infestation percentage of *Livoneca redmanii* on *Mugil cephalus* in relation to length groups (cm) at Lake Qarun during the year 2016.

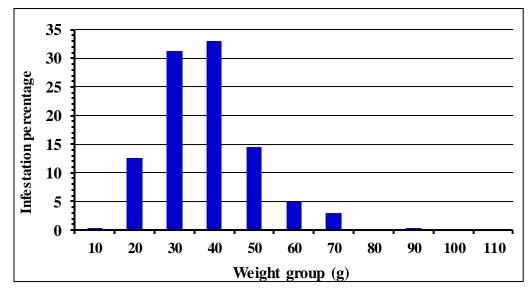


Fig. (5): Prevalence infestation rate of *Livoneca redmanii* on *Mugil cephalus* in relation to weight groups (g) at Lake Qarun during the year 2016.

Sites of infection:

After examination of 269 infested *Mugil cephalus* notice the infested fishes are mostly females. The ecto-parasitic isopods, *Livoneca redmanii* infect gills and skin of ventral and/or dorsal sides. Examination of infected host revealed that, these parasites were found attached to the host with their appendages deeply embedded in the fish muscles and anchored themselves by their hooks in the terminal appendages (**Figures**, **6-8**). Data showed that, there are 264 (98.1 %) infested by the parasite in their gills. However, the remaining five (1.9 %) infected fishes infested by the parasite in their skin, three dorsally infected and the other two ventrally infected.

Clinical signs and post mortum lesions of infested *Mugil cephalus* with *Livoneca redmanii*:

The main clinical signs examination in naturally infested *M. cephalus* showed that the form of motion slow swimming at the surface of the water debilitated with extensive mucus as well as they become off food and rubbing against hard object. Fish infested with *Livoneca redmanii* showed increased opercula movement, gathering around air source, some aggregated on the water surface and accumulated at the fresh water inlet of the pond, grasping of air at water surface, absence of escape reflex and emaciation as well as degenerative and hyperplastic changes in skin and the scale external body surface as well as sloughing (Fig.9). The investigation of *M. cephalus* revealed that bilateral and/or unilateral infection per hosted fish in gills region and the other part of the parasite protruded outside the body of the fish (Fig. 10).

After examination of post mortum lesions of infected *M. cephalus* with *Livoneca redmanii* parasites revealed that ulcer and erosion blew the gills (Fig. 11). At the site of attachment, the skin host was recognized by external abnormalities such as skin ulcers, scale less and discoloration (Fig.12).



Fig. (6): Gills of Mugil cephalus infested with Livoneca redmanii



Fig. (7): Skin infested with Livoneca redmanii (dorsal side of Mugil cephalus)



Fig. (8): Skin infested with Livoneca redmanii (ventral side of Mugil cephalus)



Fig. (9): Showing hyperplastic changes in skin and scales as well as sloughing of the infested *Mugil cephalus*.



Fig. (10): Showing two parasites in gills region and the parasite protruded outside the gills of the infested *Mugil cephalus*.



Fig. (11): Showing ulcer and erosion blew the gills of the infested Mugil cephalus



Fig. (12): Showing external abnormalities such as skin ulcers, scale less and discoloration of the infested *Mugil cephalus*.

Histopathology of infected gills:

The gills of the normal fish, *Mugil cephalus* are made up of four pairs of gill arches. Each gill arch bears a double row of gill filaments [non-respiratory or primary filaments (PF)] that carry two rows of gill lamellae [respiratory or secondary lamellae (S)]. The secondary lamellae are separated by distinct inter-lamellar spaces (ILS). The primary filament is composed of multi-layered epithelium cells (primary epithelium). Many and scattered mucous cells in the inter-lamellar epithelium were seen in between the secondary lamellae. Each secondary lamella consists of a double thin sheet of epithelial layer (secondary epithelium), separated by the centrally located pillar cell system (PSC) that supports the epithelial layer and limit blood lacunae (Fig.13).

The histopathological examination of gills on which the parasite *Livoneca redmanii* was settled, showed hyperplasia (HP) of epithelial cells in gill filament (Fig. 14). Hyperplasia of lamellar epithelium and mucous secreting cells tend to be confusion (C) between secondary gill lamellae (Fig. 15), lifting (L) of secondary lamellae (Fig. 16) and congested blood vessels (CBV) were also noticed (Fig.17).

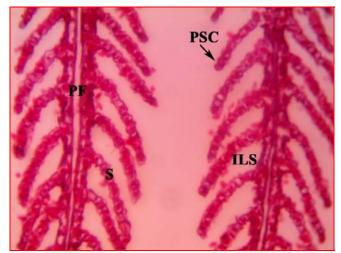


Fig. (13): Photomicrograph of T.S. in gills of the control *Mugil cephalus* showing: primary gill filaments (PF); bear double rows of the secondary lamellae (S); interlamellar space (ILS) and pillar system cells (PSC) (H & E x 400).



Fig. (14): Photomicrograph of T.S. in gills of infected *Mugil cephalus* showing hyperplasia (HP) of epithelial cells in gill filament and tend to be secondary lamellae (S) (H & E x 400).



Fig. (15): Photomicrograph of T.S. in gills of infected *Mugil cephalus* showing hyperplasia (HP) of epithelial cells and tend to be confusion (C) between secondary lamellae (S) (H & E x 100).



Fig. (16): Photomicrograph of T.S. in gills of infected *Mugil cephalus* showing lifiting (L) of secondary lamellae (S) (H & E x 100).

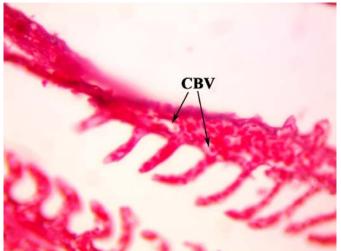


Fig. (17): Photomicrograph of T.S. in gills of infected *Mugil cephalus* showing congested blood vessels (CBV) (H & E x 100).

DISCUSSION

Some isopods recorded as parasites on many species of commercially important fishes causing significant economic losses to fisheries (Rameshkumar *et al.*, 2014), where the incidence and intensity of parasitic isopods exhibit a considerable variation (Grutter, 2003 and Cuyas *et al.*, 2004).

Isopoda are prospective the leading group of the fish crustacean parasites (Kabata, 1984). They are actually economically significant parasites as they have been identified to cause deleterious effects on cultured fish, such as stunted growth, anemia, and mortalities of fish fries and finger lings (Ravi and Raj kumar, 2007 and Ravichandran *et al.*, 2011).

In the present study, the highest infestation rate of isopod, *Livoneca redmanii*, was 46.70 % recorded on *Mugil cephalus* from Lake Qarun. It may be due to slow movement of mullet and large of surface body area to excess of infested by parasites. This rate considerably higher than that recorded by some authors in Egypt, such as 8.62 % (Badawy, 1994) from Mediterranean Sea at Port-Said, 9 % (Abd El Aal and El Ashrum, 2011) from Matroh, and Alexandria provinces, and 10.26 % (Samn *et al.*, 2014) from Mediterranean Sea, Alexandria. Also it is higher than reported by Eissa *et al.* (2012) (19 %) among Mediterranean Sea fishes and also from Suez Canal in Ismailia province (24.4 %). Mahmoud *et al.* (2016) revealed total prevalence of 32.66% with isopod infestation among *D. Labrax, S. vulgaris* and *T. zilli* from Lake Qarun. These prevalence variations might be attributed to the differences in the periods of investigation, the examined fish species and the geographical distribution of fish hosts and parasites.

Cymothoid isopods generally breed twice a year, in February and August (Aneesh *et al.*, 2013). Female parasites with eggs were accordingly observed in February and August in the present study. Therefore, the highest levels of intensity and variety of parasites were observed in summer, whereas the least infestation parasites were observed in winter. This can be explained by parasites that usually prefer the coastal area, while fish migrate to deeper waters in the winter as a result of changes of water temperatures in the sea (Er and Kayis, 2015).

In the present study, the highest infestation rate of *Livoneca redmanii* (76.20%) was recorded in *M. cephlus* from Lake Qarun during February and the lowest occurred in January (19.40%). This may be attributed to differences in geographical distribution of hosts, time of reproduction of parasites and topographical nature of Lake Qarun during different months. In addition, the increases or decreases of drainage waters in the lake which heavily loaded with wastes, salts, nutrients, pesticides, heavy metals and organics that may accumulate and contaminate the aquatic environment (Abdel-Monem, 1991 and Khalaf-Allah, 2001 & 2014). Moreover, a remarkable increase in the bacterial indicators of sewage pollution in the lake was recorded (Ibrahim and Ramzy, 2013).

Concerning sex, the infestation rates of *Livoneca redmanii* in the present study were increased in females (49.60 %) than males (42.10 %) in *M. cephalus*. It may be due to slow movement of females too easy of infested by parasites. Samn *et al.* (2014) noticed that *Nerocila bivittata* infect mostly female of *Lithognathus mormyrus* from Abu Qir Bay, Alexandria, Egypt. On the other hand, the highest infestation rate of *Livoneca redmanii* on *M. cephalus* was 60 % recorded in small fishes at length group 11.1 - 12 cm and the lowest rate (20 %) occurred in large ones at length group 21.1 - 22 cm. It may be due to the number of small fish specimens in the catch is very large compared with large ones. Also, in the present study, the highest infestation rate of

Livoneca redmanii on *M. cephalus* was 55.60 % recorded in large weight fishes. Eissa *et al.* (2012) cleared that, the infestation rate was increases with increasing in length and weight of hosted fish. The relation between fish body weights, lengths and infestation rate was significant for infested fishes.

In the present study, the ecto-parasitic isopod, *Livoneca redmanii*, infect gills, ventral side and dorsal side of *Mugil cephalus*. Noor El-Deen *et al.* (2013) mentioned that crustacean parasites, *Caligus minimus and Caligus elongate* were collected from skin of mullet fish, *Mugil cephalus* in Damietta, Egypt.

The position of attachment in ventral, dorsal, and tail sites might depend on the host's body movement. Fish swim using undulatory movements of their body and/or their paired and unpaired fins. In undulatory swimming, a backward travelling wave is generated by the sequential activation of the segmental myotomes from head to tail (Altringham and Ellerby, 1999 and Samn *et al.*, 2014). The cause of attachment at this position may be due to easier attachment at this site by the parasite or due to easier shedding of the parasite from other areas by the host (Printrakoon and Purivirojkul, 2011).

Examination of the infested fish revealed that the intensity of infestation was one or two parasites per hosted fish in gills region or attached on the skin and slight protrusion of gill cover (operculum) causing atrophy and hemorrhage at site of attachment. These may be attributed to the low respired oxygen of destructed gill epithelium which caused by feeding activity, attachment, fixation and locomotion of crustaceans. These results are in full agreement with those reported by Eissa *et al.* (2012) and Kayiş and Ceylan (2011). Who found a female *Nerocila orbignyi* between the operculum and pectoral fin of *Solea solea*. This was also evident in the present study; the free specimens of parasite are male and convert to female when attachment of host gills.

In the present study, the clinical examination of most infected fish, *M. cephalus* with *Livoneca redmanii* showed some slight abnormalities as represented as respiratory manifestation, sluggish movement, surface swimming, bulging of opercula, some fishes aggregated on the surface and accumulated at the mouth of agriculture drains and fresh water inlet of the pond. Also, fishes showed emaciation, excessive amounts of mucous covering the body surface and some fishes has scale sloughing. These results were similar to that obtained by Noor El- Deen *et al.* (2013 & 2015) and Abdel-Mawla *et al.* (2015). Excessive mucous secretion may be used to dilute the irritation and act as a defense mechanism against infestation (Yambot and Lopez, 1997). Also, the emaciation recorded in fish infested with isopods may have been a result of a reduced appetite for food or due to reduced growth rates (Nagasawa, 2004; Costello, 2009; Printrakoon and Purivirojkul, 2011 and Abdel-Latif, 2016).

In the present study, regarding the postmortem examination of most infected fishes, *M. cephalus*, with *Livoneca redmanii* revealed that increases of mucus producing cells, ulcer and erosion in the gills. The skin host was recognized by external abnormalities such as skin ulcers, scale less and discoloration. It may be attributed to harmful effect of parasites. These results were similar to that obtained by Eissa (2004) and Noor El-Deen *et al.* (2013).

In the present study, the highest percentage of infested fish by ectoparasitic isopods, *Livoneca redmanii*, was recorded in gills region of *M. cephalus* (98.1 %). *Livoneca redmanii*, which was isolated from the gills of the infested fishes are known to consume on gill tissues, which can cause various harm-sparticularly emaciation of affected fish. These results may be due to the adult isopods are heamatophagus (i.e., feed on blood) and cause anemia. In addition, those parasites attached to the gills can

seriously reduce the respiratory surface via atrophy of the gills on which they are attached (Horton and Okamura, 2001; Manera and Dezfuli, 2003; Mladineo, 2003; Ravi and Rajkumar, 2007; Toksen *et al.*, 2008 and Kayis and Ceylan, 2011).

In the present study, the histopathological alterations in the fish gills infested with *Livoneca redmanii* showed hyperplasia of epithelial cells in gill filament, hyperplasia of lamellar epithelium and mucous secreting cells tend to be confusion between secondary gill lamellae, lifting of secondary lamellae and congested blood vessels were also noticed. These findings were similar to that reported by Thatcher *et al.* (2003), Noor El-Deen (2007), Toksen *et al.* (2008), Noor El-Dean *et al.* (2012), Rameshkumar *et al.* (2013), Youssef *et al.* (2014) and Abdel-Mawla *et al.* (2015). The detected deleterious impacts such as confusion between secondary gill lamellae, lifting and congested blood vessels might be attributed to the pressure of the parasite feeding and its direct contact with the large sized isopod species (Kabata, 1970 and Abdel-Latif, 2016). This also was evident in the present study.

It is now clear that cymothoid isopod, *Livoneca redmanii* was causes economic loss associated with reduced fish growth, high mortality and marked drop in fish production from Lake Qarun.

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