



Prevalence of *Lernanthropus Kroyeri* in Seabass (*Dicentrarchus Labrax*) and spotted seabass (*Dicentrarchus Punctatus*) from Suez Canal, Egypt

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Abstract

Copepod parasites (*Lernanthropus Kroyeri*) were found in Suez Canal, Egypt from September 2017 to the end of August 2018. The copepod parasite infested the gills of commercially important fish hosts including, seabass (*D. labrax*) and spotted seabass (*D. punctatus*). The total prevalence was 64.25%. The winter and summer displayed the highest seasonal prevalence in seabass and spotted seabass respectively. Host fishes provoked moderate to severe pathognomonic abnormalities as signs of asphyxia, opened mouth, Opercular bulging, marbling appearance of the gills and heavy infestation of the copepods in between gill filaments. The relation inter between fish body weight, length and infestation rate were studied. The histopathological manifestation was recorded also as erosions, necrosis of the secondary lamellae and atrophy at the site of parasitic attachment.

Keywords: *Dicentrarchus labrax* (seabass), *Dicentrarchus punctatus* (spotted seabass), *Lernanthropus kroyeri*, prevalence, histopathology

Introduction

Fish involve the primary spot at the highest point of the human nourishment list, as they are viewed as a major source of proteins, especially marine fish which contain a lot of Polyunsaturated unsaturated fatty acids (PUFAs) that assume a role in decreasing the cholesterol level in blood, give protection from arteriosclerosis and a heart diseases (Van Bilsen, 2014) [28]. The two *Dicentrarchus* species belong to Moronidae family which are common in the Mediterranean Sea, have similar morphologies in their early stages, and differ when *D. labrax* grows to a greater commercial length than *D. punctatus* and *D. punctatus* has black spots scattered over its body (Bonhomme *et al.*, 2002) [4]. Fish may be affected by different species of parasites causing major losses to fish fry and fingerlings and responsible for deterioration of the general health condition of the fish and in turn, fish become susceptible to various diseases of other factors. This is leading to hazardous economical losses (Eissa, 2002) [10]. Parasitic diseases affecting marine fishes are abundant and they cause high losses in aquaculture of seabasses in Egypt (Khalil *et al.*, 2014) [14]. Among marine fish parasites, nearly 25% are crustaceans, mainly isopoda, branchiura and copepod (Eiras *et al.*, 2000) [6]. Parasitic copepods are commonly found in cultured and wild marine fishes (Jones *et al.*, 2012; Ramesh-kumar and Ravichandran 2013) [13, 22]. Disease outbreaks and mortalities caused by *L. kroyeri* are frequently observed in *D. labrax* with prevalence of 35% (Manera and Dezfuli, 2003) while (Noor El-Deen *et al.*, 2013) [19] recorded 20%. This parasite present attached to the gill filaments of sea bass from Kafrelsheikh governorate, Egypt with prevalence 18% (Zaid *et al.*, 2018) [31]. *Lernanthropus* causing histopathological effects as erosion and necrosis of gill filaments of the seabass (Manera and Dezfuli, 2003) [18]. The objectives were decided to throw the light on, the clinical picture, seasonal prevalence of *L.*

kroyeri affecting sea bass and spotted sea bass. Besides, the relation between fish body length and weight with infestation and the histopathological manifestations were carried out.

Materials and Methods

a. Fishes

A total number of 193 alive fish (92 *Dicentrarchus labrax* and 101 *Dicentrarchus punctatus*) of different body weights were randomly collected between September 2017 to the end of August 2018 from Suez Canal in Ismailia Governorate, Egypt. The collected fishes were taken alive in tanks containing 1/3 of its volume water from the site of capture where the remaining volume was filled with air.

Table 1: Showing number of examined fish species

Fish species	Common name	No. of exam. fish	Arabic name
<i>D. labrax</i>	Sea bass	92	Karous
<i>D. punctatus</i>	Spotted sea bass	101	Nokt
Total		193	

Table 2: Showing number of examined fishes in different seasons:

Season Fish type	Autumn	Winter	Spring	Summer	
<i>D. labrax</i>	15	17	27	33	92
<i>D. punctatus</i>	26	25	19	31	101
Total	41	42	46	64	193

b. Clinical picture

First, body weight and length of the examined fishes were recorded, then clinical examination was done on the both fishes, (live or freshly dead ones). Fish specimens under investigation were grossly examined for determination of any external parasite and any clinical abnormalities. The PM examination was performed on all fish according to (Amlacker, 1970) [1].

c. Parasitological examination

1. **Macroscopic examination:** Macroscopic examination was done by naked eyes and hand lens to detect any abnormalities, the operculi removed with scissors, exposing gills, dissected separately and examined with a microscopic magnified lens.
 2. **Microscopic examination:** Gill filaments were transferred to slides with a drop of marine water and cover slip to prevent drying and examined microscopically (Lucky, 1977) [16].
- d. **Preparation of permanent specimens for identification** The detected Copepods collected by dissecting needle and a fine brush then kept in a small vial, washed by distilled water. They fixed in 3% formalin and preserved in equal amount of 70% alcohol – 5% glycerin in test tube and constant amounts prepared by passing in descending grades of alcohol (70%, 50%, 30%), cleared in glycerin and mounted in glycerin-gelatin, according to (Lucky, 1977) [16].
- e. **Histopathological examination:** gills were fixed in 10% neutral formaldehyde solution for pathologic examination. Tissue samples were treated and embedded in paraffin. Tissue sections 4-6 μ in width were stained with haematoxyline-eosin (HxE) and examined under light microscope (Roberts, 2001) [23].

Results

1. Clinical picture of naturally infested fishes

The clinical picture in the naturally infested fishes with copepods collected from Suez Canal area, showed moderate to severe pathognomonic clinical abnormalities especially in case of heavy infestations. The clinical signs were haemorrhagic areas on the body surface of the infested fish especially around the mouth, on the gill cover and, at the base of fins and opened mouth. Opercula were bulging with swallowing the atmospheric air (surface breathing) (Photo 1). Gills of *D. labrax* and *D. punctatus* showed a marbling (mosaic) appearance (areas of redness and paleness) (Photo 2). Gill tips were stacked in some areas with petechial haemorrhages were seen in the gills of some fishes. Also, there were excessive mucous secretion covered the gills. *Lernanthropus kroyeri* were visible by naked eyes as black lines between the gill filaments in case of heavy infestations, increased mucus secretion, haemorrhagic lines and pale gill filaments. In addition, the site of the female parasite was mainly found in the gill filaments of the second gill arch of *D. labrax* and usually absent in the first one (Plate 1).



Photo 1: *D. labrax* displaying hemorrhagic areas on the body surface especially around the mouth, on the gill cover and at base of fins.



Photo 2: Gill arch of *D. labrax* in the petri dish showing a marbling appearance (areas of congestion and paleness)

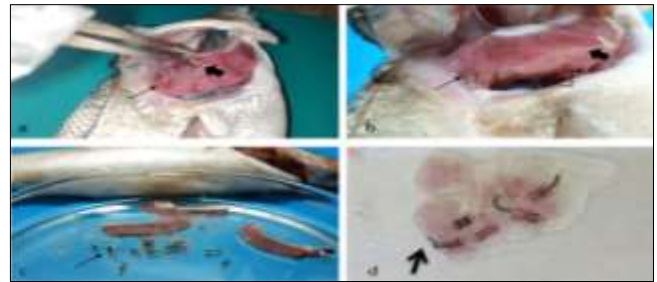


Plate 1: (a) and (b) *D. labrax* exhibiting heavy infestation of *Lernanthropus kroyeri* (arrow) detected between the gill filaments as black lines (arrow), stacking of tips gill filaments (arrowhead) and excessive mucus (elbow arrow). (c) Gills in the petri dish showing *Lernanthropus kroyeri* (arrow). (d) *Lernanthropus kroyeri* with part of gill filaments squashed gently between 2 slides (arrow).

Meanwhile, the infested *D. punctatus* showed *Lernanthropus kroyeri* between the gill filaments as black lines with observation of petechial haemorrhage, mosaic appearance, excessive mucus secretion and pale gill filaments (plate 2).



Plate 2: (a) *D. punctatus* showing, (b) Heavy infestation of *Lernanthropus kroyeri* (arrow) detected between the gill filaments as black lines and stacking of tips of gill filaments. (c) *Lernanthropus kroyeri* (arrow) in the petri dish in-between pale gills.

2. Results of parasitological examination

Lernanthropus kroyeri. 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. Cephalothorax bears five pairs of cephalic appendages (antennules, antennae, maxillules, maxillae, and mandibles) and three pairs of thoracic appendages (maxillipeds and two pair of legs).

The trunk, consisting of posterior part of the thorax and the abdomen, bears two or three pairs of legs according to sex, the genito-abdominal complex, and caudal rami. (Plate 3 and 4).

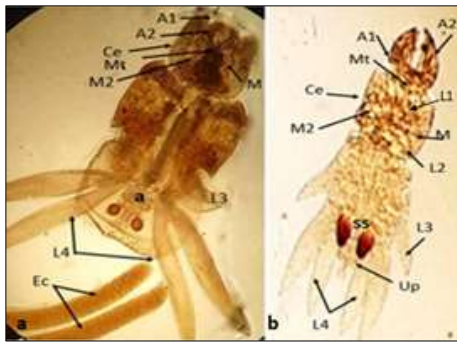


Plate 25: *Lernaanthropus Kroyeri* isolated from *D. labrax* a: female copepod. c: whole male *Lernaanthropus Kroyeri* showing its hard claws. A1: first antenna; A2: second antenna; Mt: mouth tube; M: maxilliped; M2: second maxilla; Ce: cephalothorax; L1: 1st thoracic leg; L2: 2nd leg; L3: 3rd leg; L4: 4th leg; a: abdomen; SS: spermatophore sac; Up: Uropods; Ec: egg sac.

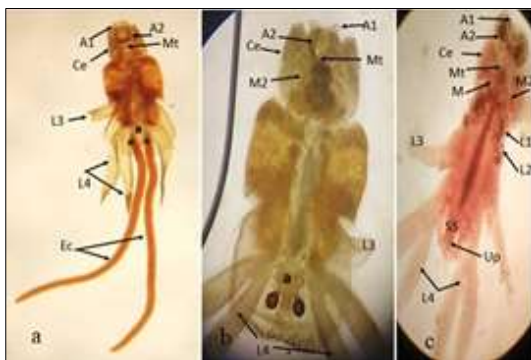


Plate 26: *Lernaanthropus Kroyeri* isolated from *D. punctatus*. a:

whole female copepod. b: Anterior part of female copepod. c: whole male *Lernaanthropus Kroyeri* showing its hard claws. A1: first antenna; A2: second antenna; Mt: mouth tube; M: maxilliped; M2: second maxilla; Ce: cephalothorax; L1: 1st thoracic leg; L2: 2nd leg; L3: 3rd leg; L4: 4th leg; a: abdomen; SS: spermatophore sac; Up: Uropods; Ec: egg sac.

3. Prevalence of *Lernaanthropus kroyeri* infestation among the examined fishes

Table (3 and 4) and their charts show the total and seasonal prevalence of *Lernaanthropus kroyeri* infestations among the examined *D. labrax* and *D. punctatus*. Tables (5&7) shows Prevalence of

Lernaanthropus kroyeri infestations in relation to length and body weights among *D. labrax*. Tables (6&8) shows Prevalence of *Lernaanthropus kroyeri* infestations in relation to length and body weights among *D. punctatus*.

Table 3: Total prevalence of *Lernaanthropus kroyeri* infestations in examined fish species

Fish Species	No. of examined Fish	No. of infested Fish	Prevalence %
<i>Dicentrarchus labrax</i>	92	57	61.96
<i>Dicentrarchus punctatus</i>	101	67	66.34
Total	193	124	64.25

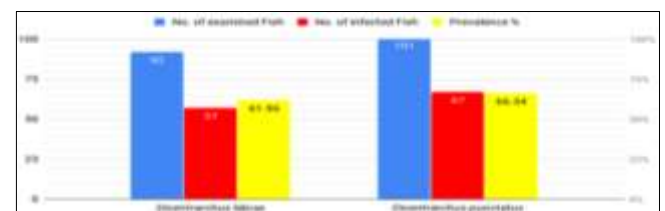


Chart 1: Prevalence of *Lernaanthropus kroyeri* among examined fish species.

Table 4: Seasonal prevalence of *Lernaanthropus kroyeri* infestation among the examined fishes

Fish type Season	<i>D. labrax</i>	<i>D. punctatus</i>	Total (n=193)	Total infested	Prevalence %
	No. (%) of infested Fish	No. (%) of infested Fish			
Autumn n (15,26)	10 (66.67%)	17 (65.38%)	41	27	65.85%
Winter n (17,25)	15 (88.24%)	17 (68%)	42	32	76.19%
Spring n (27,19)	14 (51.85%)	11 (57.89%)	46	25	54.35%
Summer n (33,31)	18 (54.55%)	22 (70.97%)	64	40	62.5%
Total n (92,101)	57 (61.96%)	67 (66.34%)	193	124	64.25%

*n: number of examined fishes (*D. labrax*, *D. punctatus*, *S. aurata*) respectively.

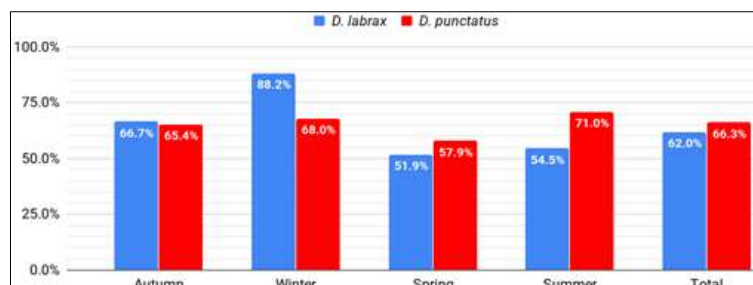


Chart 2: Seasonal prevalence of *Lernaanthropus kroyeri* among examined fishes.

Table 5: Prevalence of *Lernaanthropus kroyeri* infestations in relation to body length among *D. labrax*

Fish length (cm)	No. of Examined Fish	Lernaanthropus infestation
		*No. infested (%)
10-20	18	8 (44.4%)
20-30	40	23 (57.5%)

30-40	28	23 (82.1%)
40-50	6	3 (50%)
Total	92	57 (62%)

*No.: number of infested fish and the prevalence.

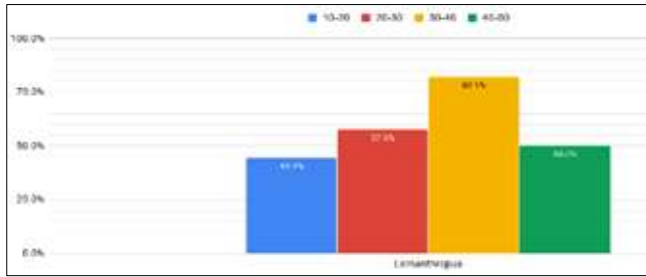


Chart 3: Prevalence of *Lernanthropus kroyeri* infestations in relation to length among *D. labrax*.

Table 6: Prevalence of *Lernanthropus kroyeri* infestations in relation to length among *D. punctatus*

Fish length (cm)	No. of examined Fish	Lernanth. infestation
		*No. infested (%)
10-15	21	12 (57.14%)
15-20	56	36 (64.29%)
20-25	24	19 (79.17%)
Total	101	67 (66.34%)

*No.: number of infested fish and the prevalence.



Chart 6: Prevalence of *Lernanthropus kroyeri* infestations in relation to body weight among *D. punctatus*.

VI. Histopathological examination of naturally infested fishes

The gills of *D. labrax* naturally infested with *Lernanthropus kroyeri* showed severe erosion, desquamation and necrosis of the secondary lamellae. Severe compression and atrophy at the site of parasitic attachments were also seen. Hyperplasia and adhesion of secondary lamellae along with severe leukocytic infiltrations mainly with eosinophils and lymphocytes (Plate 36).

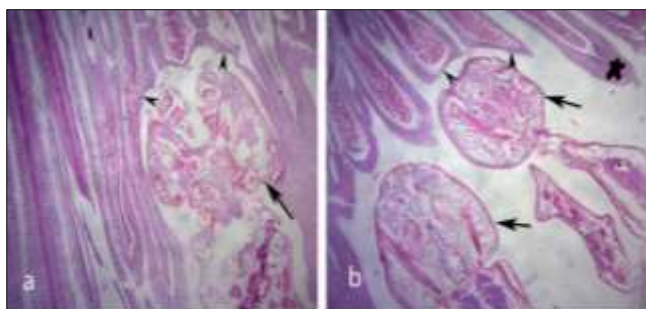


Plate 36: Gills of *D. labrax* infested with *Lernanthropus kroyeri* (arrows) showing sections of the parasite *Lernanthropus kroyeri* that causing severe atrophy of the gill lamellae (arrow heads) along with hyperplasia of gill epithelial cells admixed with inflammatory cells. (asterisk). H&E. X 100.

Moreover, the gills of *D. punctatus* infested with *Lernanthropus kroyeri* and showed massive degeneration of gill lamellae necrosis and atrophy (Plate 42).

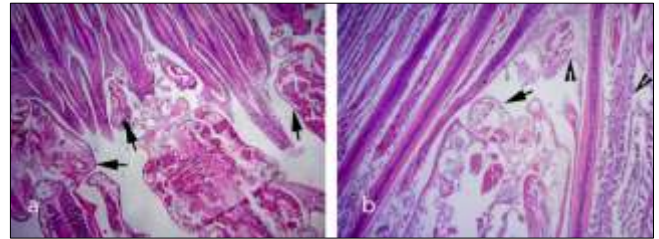


Plate 42: Gills of *D. punctatus* infested with *Lernanthropus kroyeri* (arrows) and showing massive degeneration of gill lamellae necrosis and atrophy (arrow heads). H&E. X 100.

Discussion

This study revealed haemorrhagic areas on the body surface especially around the mouth, on the gill cover and at the base of fins of *D. labrax*, these signs go hand in hand with the findings of Eissa *et al.* (2012)^[10] and El-Gohary and Diab A. M. (2014)^[9]. Furthermore, these lesions may be assigned to severe irritation caused by movement, feeding habits, rigid attachment of crustaceans by their claws and mucous act as a defence mechanism against the infestation and to reduce the irritation (Heba Abdel-Mawla and El-Ekiaby, 2012)^[11]. Regarding *Lernanthropus kroyeri* were visible by naked eyes in heavy infestations of *D. labrax* and *D. punctatus* forming black lines between the gill filaments in case of heavy infestations, increased mucus secretion, haemorrhagic lines and anaemic gill filaments. This result agreed with Woo and Leatherland (2006)^[29] and Yardimci and Pekmezci (2012)^[30].

Furthermore, there was a notice about the site of the female parasite where mainly found in the gill filaments of the second gill arch of *D. labrax* and usually absent in the first one, this result similar with Toksen (2007)^[26] who recorded that the female parasite choose the deep place between the hemibranchs of the second gill arch. Unusual, they were observed in gill filaments of first and third gill arch, but male parasites were seen on the posterior hemibranchs next to females.

Concerning *Lernanthropus Kroyeri* isolated from the gills of *D. labrax*, this result agreed with Manera and Dezfuli (2003)^[18], Toksen (2007)^[26], Ola Abu Samak and Ashraf (2008)^[21], Eissa *et al.*^[10]

(2012) and Yardimci and Pekmezci (2012)^[30] but differ from that obtained by Noor El-Deen *et al.* (2013)^[19] and Dawlat Hassanin (2016)^[5] who isolated the same parasite from other fish species such as *Mugil cephalus* and *Moolgarda seheli*. *Lernanthropus Kroyeri* isolated from the gills of *D. punctatus*, this result agreed with that obtained by Eman El-Boghdady *et al.* (2015)^[10] who isolated *Lernanthropus Kroyeri* isolated from *D. labrax* and *D. punctatus*. Regarding to isolation of *Lernanthropus Kroyeri* from *D. labrax* and *D. punctatus* in parallel to Luque and Paraguassu (2003)^[17] and Sharp *et al.* (2003)^[24] that many of *Lernanthropus sp.* are parasitic on many species of fish belonging to one or on

various genera of one family. The morphological features of *Lernanthropus Kroyeri* agreed with that obtained by Toksen (2008)^[27].

Regarding the total prevalence of *Lernanthropus kroyeri* was 64.25% this result was greater than that obtained by Noor El-Deen *et al.* (2013)^[19] which was 20% and Zaid *et al.* (2018)^[31] which was 18%, conversely, it is lower than that obtained by Toksen (2007)^[26] which was 100 % and Aneesh *et al.* (2014)^[24] as it was 81.4%. This disparity may be due to the locality from which the fish obtained and the differences in type and size of the fish. Moreover, the infestation rate was recorded in *D. punctatus* 66.34%, this was higher than that obtained by Bahri *et al.* (2002)^[3] as it was 25%. Eman El-Boghdady *et al.* (2015)^[10] which was 41%. Meanwhile, in *D. labrax* was 61.96%, this was higher than that obtained by Bahri *et al.* (2002)^[3] as it was 12.9% and Oktener *et al.* (2010) which was 41%, however, the result was lower than that obtained by Yardimci and Pekmezci (2012)^[30] which was 100%. Furthermore, *L. kroyeri* was more frequent on spotted bass than on common seabass, this result in parallel to that obtained by Bahri *et al.* (2002)^[3].

In this study, seasonal prevalence of *Lernanthropus kroyeri* infestation in *D. labrax* was highest (88.24%) in winter and lowest (51.85%) in spring, this disagreed with result obtained by Ola Abu Samak and Ashraf (2008)^[21] as it was high in (42.5%) in autumn and lowest (7.5%) in spring. Meanwhile, in *D. punctatus* in this study, it was the highest (70.97%) in summer and lowest (57.89%) in spring and this disagreed with the result obtained by Eman El-Boghdady *et al.* (2015)^[10] as it was maximum value (80%) in autumn.

In the present study, *D. labrax* showed positive correlation between prevalence of *Lernanthropus* at length of fish 10-40 cm then decreased at length 40-50 cm. Also, In *D. punctatus*, prevalence of *Lernanthropus* infestations showed positive correlation with fish length up to length 10-25 cm, this result confirmed by the result obtained by Eissa *et al.* (2012)^[10] as there was positive correlation with increase in length and agreed with Dawlat Hassanin (2016)^[5] as prevalence of crustacean infestations showed positive correlation with increase with length. Meanwhile, *D. punctatus* showed positive correlation between weight of fish and prevalence of *Lernanthropus* infestations up to weight <50-150g then number of fish decreased at weight 150-200g. This result agreed with Timi and Lanfranchi (2006)^[25] as the prevalence of copepode increased with host weight up to fish with intermediate weight then it decreased because changes in size of gill filaments affect their attachment and can be easily detached by respiratory currents.

Regarding to the histopathological changes of the gills of *D. labrax* and *D. punctatus* naturally infested with *Lernanthropus kroyeri* showed sections of the parasite, severe erosion, desquamation and necrosis of the secondary lamellae. Severe compression and atrophy at the site of parasitic attachments were also seen. Hyperplasia and adhesion of secondary lamellae along with severe leukocytic infiltrations mainly with eosinophils and lymphocytes, this result in accordance to Manera and Dezfuli (2003)^[18], Korun and Tepecik (2005)^[15], Toksen (2007)^[26], Jithendran *et al.* (2008)^[12], Yardimci and Pekmezci (2012)^[30]. These lesions may be attributed to fixation of crustacean parasites with their claws activity and the severe irritation caused by feeding activity, movement which lead to asphyxia. So, in conclusion the variation between this study and other studies might be due to type of the fish host, feeding habits, swimming habits,

length and weight of the examined fishes, time of collection and the site from which fish samples were obtained.

Conclusion

Through September 2017 to the end of August 2018, the study was achieved to record *Lernanthropus kroyeri* of *D. labrax* and *D. punctatus* from Suez Canal in Ismailia Province, Egypt. This study proved the prevalence of the parasite, seasonal prevalence, the relation between fish body length and weight with infestation and the serious lesions and necrosis of the primary and secondary lamellae of the gills of the examined fishes that caused deterioration of their health.

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