

## Studies on Crustacean Diseases of *Seabass* and *White grouper* fishes in Port Said Governorate

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### Abstract

The present study was designed to investigate the parasitic crustaceans affecting Seabass (*Dicentrarchus labrax*) and White grouper (*Epinephelus aeneus*) which collected seasonally from different areas of Mediterranean sea region (Port Said province). No pathognomic signs were detected in the infested fishes. Some revealed signs and P.M. lesions as sluggish movement, bulging of opercula, marbling (mosaic) appearance. Gills showed areas of thickened mucus and petechial hemorrhages and emaciation. The crustacean parasites were identified as copepods of *Lernanthropus kroyeri* and *Caligus minimus* in *D. labrax* and isopods of *Praniza* larva in *E. aeneus*. The total prevalence of crustacean infestations among seabass *D. labrax* was (36%) while in White grouper *E. aeneus* was (18%). The autumn and spring displayed the highest seasonal prevalence in seabass and grouper respectively. The relation between fish body weights, lengths and infestation rate were also studied.

### Introduction

Nowadays, marine fishes take the upper hand as a good animal protein for man, animal and birds. It is usually advise both sick and healthy man to eat fish as they contain large amount of unsaturated fatty acids which help in minimizing the cholesterol level in the blood and protect the eaters from arteriosclerosis, heart disease and cancer of colon. Marine fishes are rich in trace elements (iodine and phosphorous), the trace elements are essential for cell metabolism (*Hisck, 1987*). Parasitic infestations represent the majority of the known

infectious diseases affecting fish (*Eissa, 2002; Ragias et al., 2004; Timi and Lanfranchi, 2006 and Noga, 2010*). The present study was directed towards further understanding of *Dicentrarchus labrax* and *Epinephelus aeneus* in Mediterranean sea region (Port Said province). This study was decided to detect the clinical picture, P.M. lesions, total and seasonal prevalence of the parasitic crustacean diseases affecting each fish species and the infestation rates in relation to body lengths and body weights.

## Materials and methods

### Fishes:

A total number of 200 alive fish (100 *Dicentrarchus labrax* and 100 *Epinephelus aeneus*) of different body weights were randomly collected from Mediterranean sea in Port Said. The collected fishes were taken alive in large tanks surrounded with ice.

### 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)*.

### Parasitological examination:

#### 1. Macroscopic examination:

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

#### 2. 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 marine water and cover slip to prevent drying and examined microscopically (*Lucky, 1977*).

#### 3- Smear preparations, permanent slides:

The attached crustaceans were collected. They were recovered, 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 amount of 70% alcohol-5% glycerin in test tubes. Permanent amounts were prepared by passage in ascending grades of glycerin alcohol (30,50,70, 80,90 and 100%), cleared in glycerin and mounted in glycerin-gelatin, according to *Lucky (1977)* then examined microscopically.

## Results

### Clinical picture:

The clinical picture in the naturally infested fishes (*Dicentrarchus labrax* and *Epinephelus aeneus*) were represented as distress, surface swimming, excessive mucus production, sluggish movement, emaciation and rubbing the body against hard objects. Opercula were bulging with gulping the atmospheric air (surface breathing). Gills of *D. labrax* showed a marbling (mosaic) appearance (areas of congestion and paleness). Excessive mucous secretion, paleness was seen in gills of some fishes. Gills showed areas of thickened mucus, petechial hemorrhages, gill tips were stuck with grayish coloration and necrosis. The parasites were visible by naked eyes in form of black lines between the gill filaments. *E.*

*aeneus* had either pinpoint ulcerative lesions in the buccal area. Palate of upper Jaw and tongue of the infested fishes showed severe multifocal hemorrhagic spots. Some parasites were found in the inner surface of the operculum. Infested *E. aeneus* showed isopoda in the buccal cavity with sliminess, ulcer and erosion in the area of attachment (Plate 1).

### **Results of parasitological examination:**

#### **1- Crustaceans isolated from *Dicentrarchus labrax*:**

*Lernanthropus kroyeri* Beneden, 1851. The body of female and male isolated copepods appeared elongate in both sexes, the cephalon and the first thoracic segment were fused to form a cephalothorax which was slightly wider than long. The cephalothorax was narrower anteriorly with a dorsal shield curved ventrally on each side in female end and flat in male. The cephalothorax was divided into a large posterior thoracic plate and a small anterior cephalic plate by two dorsolateral prominent sutures. The thoracic appendages are larger than the first and second thoracic legs which have hand fingers-like spines in the end. Females characterized by egg-strings which were seen clearly on the gills (Plate 2).

*Caligus minimus*, (Otto, 1821). It was isolated from the gills and inner surface of the operculum of *Dicentrarchus labrax*. The tagma is

longer than the thoracic zone of shield. The posterior segment of the cephalothorax is joined with an apron which includes third leg and the tagma. The genital segment and fourth leg-bearing segment of the thorax are called the genital complex. In the genital segment, intestine, immature eggs, and oviduct channel are also founded. Abdomen is the last part of *C. minimus*, it contained abdomen and caudal rami. In addition, egg column, mature and immature eggs were identified. The shape of the eggs is cylindrically flattened (Plate 3).

#### **2- Isopod parasite isolated from the buccal cavity of *Epinephelus aeneus*:**

Third stage pranzia larva (*Gnathia pilosus*). Body length was 1.8–3 mm. Cephalosome posterior margin was wider, concave than anterior margin, wider at the base, lateral margin was convex, few setae were present on dorsal posterior cephalon and posterior margin was straight, triangular-shaped cephalosome. Compound eyes were large, triangular-shaped, bulbous. Medio-anterior margin appear straight with lateral concave excavations (Plate 4).

#### **Prevalence of crustacean infestation among the examined fishes:**

Tables (1&2) show total and seasonal prevalence of crustacean infestations among seabass *D. labrax* and *E. aeneus* respectively.

Tables (3&5) shows Prevalence of the recorded crustacean infestations in relation to length and body weights among *D. labrax*. Tables

(4&6) shows Prevalence of the recorded crustacean infestations in relation to length and body weights among *E. aeneus*.

**Table (1): Total and seasonal prevalence of crustacean infestations in seabass *D. labrax***

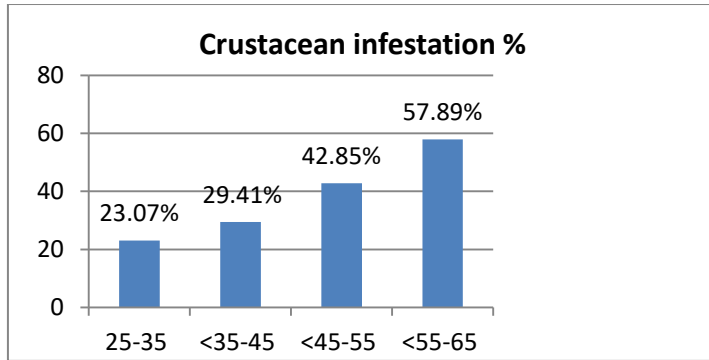
Season	No. of examined Fish	No. infested with <i>Caligus minimus</i>	No. infested with <i>Lernanthropus Kroyeri</i>	No. infested with <i>Caligus minimus</i> + <i>Lernanthropus Kroyeri</i>	No. (%) of infested fish
Autumn	25	10	5	6	21 (84)
Winter	25	0	0	0	0 (0)
Spring	25	3	0	0	3 (12)
Summer	25	6	3	3	12 (48)
Total	100	19 (19)	8(8)	9(9)	36 (36)

**Table (2): Total and seasonal prevalence of crustacean infestations in white grouper *E. aeneus***

Season	No of examined fish	No. of infested fish with praniza larva	(%) of infested fish
Autumn	25	0	0
Winter	25	3	12
Spring	25	15	60
Summer	25	0	0
Total	100	18	18

**Table (3): Prevalence of the recorded crustacean infestations in relation to length in *D. labrax***

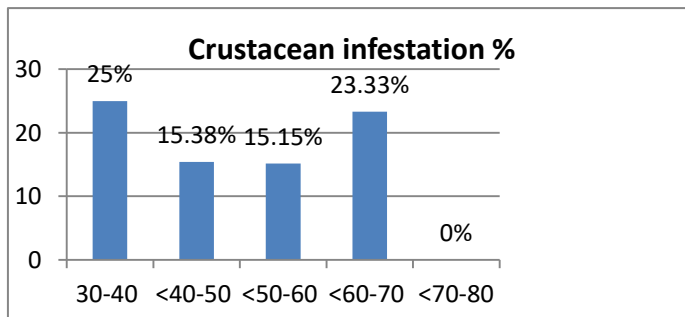
Fish length (cm)	No. examined	Crustacean infestation	
		No. infested	%
25-35	26	6	23.07
>35-45	34	10	29.41
>45-55	21	9	42.85
>55-65	19	11	57.89
Total	100	36	36



**Fig (1):** Shoening prevalence of the recorded crustacean infestations in relation to length in *D. labrax*

**Table (4):** Prevalence of the recorded crustacean infestations in relation to length in white grouper *E. aeneus*

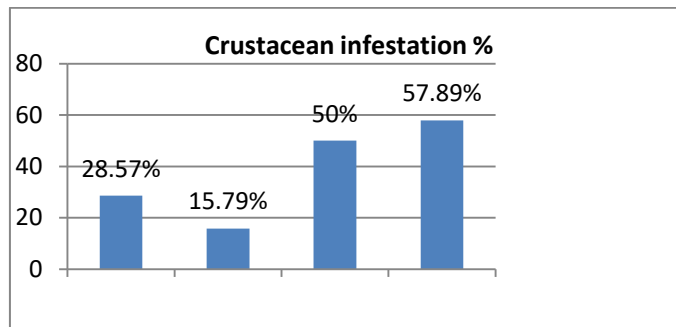
Fish length (cm)	No. examined	Crustacean infestation	
		No. infested	%
30-40	16	4	25
>40-50	13	2	15.38
>50-60	33	5	15.15
>60-70	30	7	23.33
>70-80	8	0	0
Total	100	18	18



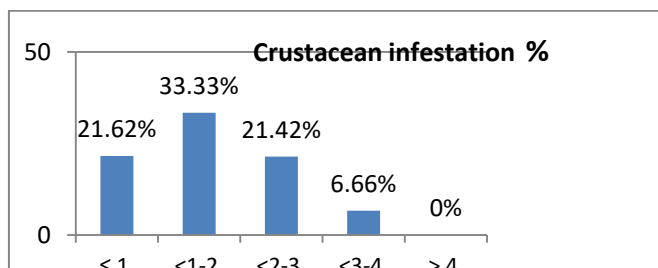
**Fig (2):** Shoening prevalence of the recorded crustacean infestations in relation to length in white grouper *E. aeneus*

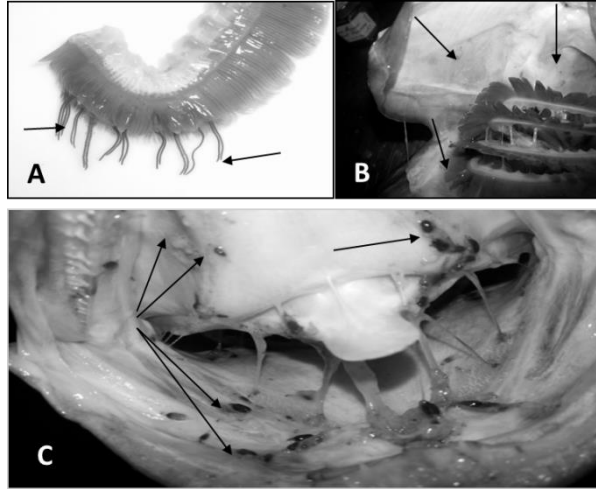
**Table (5):** Prevalence of the recorded crustacean infestations in relation to body weights in *D. labrax*

Fish body weight (g)	No. examined	Crustacean infestation	
		No. infested	%
150-650	42	12	28.57
>650-1150	19	3	15.79
>1150-1650	20	10	50
>1650-2150	19	11	57.89
Total	100	36	36

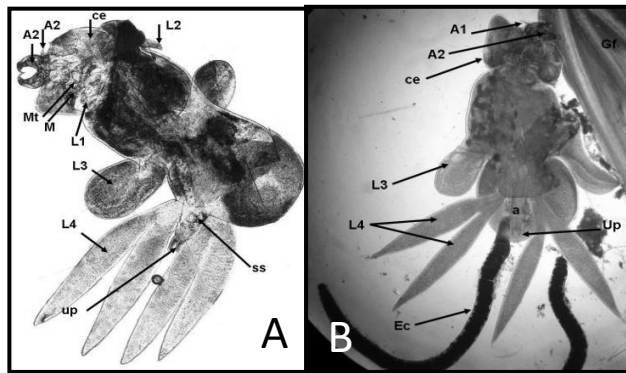
**Fig (3):** Shoeing prevalence of the recorded crustacean infestations in relation to body weights in *D. labrax***Table (6):** Prevalence of the recorded crustacean infestations in relation to body weights in white grouper *E. aeneus*

Fish body weight (kg)	No. examined	Crustacean infestation	
		No. infested	%
≤ 1	37	8	21.62
>1-2	9	3	33.33
>2-3	28	6	21.42
>3-4	15	1	6.66
> 4	11	0	0
Total	100	18	18

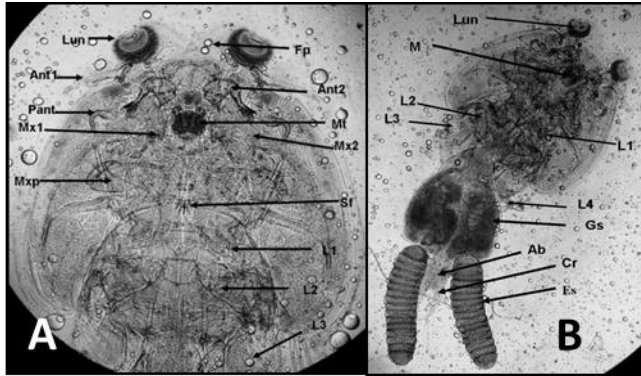
**Fig (4):** Shoeing prevalence of the recorded crustacean infestations in relation to body weights in white grouper *E. aeneus*



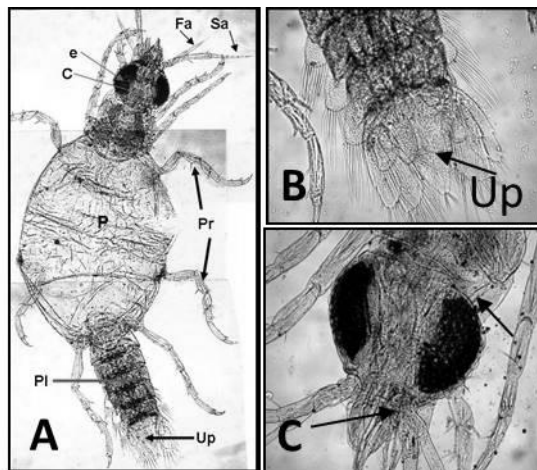
**Plate (1):** A. Gills of *D. labrax* showing black lines between the gill filaments (*Lernanthropus kroyeri*) B. Operculum of *E. aeneus* showing infestation with *Pranzia* larva (arrows) C. Mouth cavity of *E. aeneus* showing heavy infestations with *Pranzia* larva (arrows) with multifocal haemorrhagic spots.



**Plate (2):** A. Male *Lernanthropus kroyeri*: A1= First antenna, A2= Second antenna, Ce= Cephalothorax, L1= first thoracic leg; L3= Third leg; L4= fourth legs; Up= Uropods; Mt= mouth tube; M= maxilliped; SS= spermatophore sac, B. Female *L. kroyeri*: A1= First antenna, A2= Second antenna, Ce= Cephalothorax, Up= Uropods, a= abdomen, Es= Egg sac, Gf= gill filaments.



**Plate (3):** A. *Caligus minimus* : Cephalothorax ; Fp= Frontal plate; Lun = lunule; Ant1 = First antenna; Ant2= Second antenna; Mt = mouth tube; Pant = Postantennary process; Mx1 = First maxilla; max2 = Secondmaxilla; Mxp = maxilliped; Sf = Sternal furca; L1 = First leg; L2 = Second leg; L3 = third leg. B. *Caligus minimus*: Whole female parasite: Lun= Lunules; M = Mouth cone; L1 = First leg; L2 = Second leg; L3 = Third leg; L4 = Fourth leg; Gs = Genital segment; Ab = Abdomen; Cr = Caudal rami; Es = Egg sac.



**Plate (4):** A. *Pranzia* larva: Fa: First antenna; Sa: second antenna; e: eye; c: cephalon; P: Pilion; Pl: pletelson; Up: uropods; Pr: periopodes, B. Up: uropod, C. e: eye; c: cephalo

### Discussion

The present study deals with most prevailing crustacean parasitic

diseases among naturally infested seabass (*Dicentrarchus labrax*) and white grouper (*Epinephelus aeneus*)



which were caught from different sites of Mediterranean Sea in relation to the seasonal prevalence. In this work, the main clinical signs appeared in infested *D. labrax* and *E. aeneus* with crustacean infestations were distress, surface swimming, increase mucus production, sluggish movement, emaciation and rubbing the body against sides of aquaria and hard objects to remove the irritation which happened by the parasites. Opercula were slightly bulging. Fishes were surface breathing (gathered at water surface) with gulping the oxygen and atmospheric air. These results are in agreement with those reported by *Andrews et al. (1988)*, *Poynton et al. (1997)*, *Ragias et al. (2004)*, *Eissa et al. (2012)*, *Kua et al. (2012)* and *Mohamed et al. (2015)*.

Parasitic copepods feed on epithelial tissue, host mucus and blood. their feeding activities and attachment seem to be responsible for disease development. Generally, the relationship between the number of parasitic copepods and the severity of the disease is depend on; age and fish size, the general stage of fish health and the species of copepod and the developmental stages (*Pike and Wadsworth, 2000*).

Regarding the postmortem examination of the infested *D. labrax*, it was revealed (Marbling appearance) areas of paler and congestion of gills, increase mucus secretions and gill tips were sticked

with grayish coloration and necrosis. These results may attributed to the irritation which happened due to movement and feeding activity of the parasites. Increase mucus secretion result to dilute the irritation in addition to act as a defense mechanism against the infestation. In some cases, the parasites were visible by naked eyes in form of black lines between the gill filaments. These signs agreed with those reported by *Eissa et al. (2012)*.

This study showed that the mouth cavity of *Epinephelus aeneus* appeared with pinpoint or dispersed ulcerative lesions, sliminess and erosion in the area of attachment inside the buccal area. Palate of upper Jaw and tongue showed multifocal hemorrhagic spots. These results may be attributed to feeding activity, movement, severe irritation and fixation of third stage pranzia larva (*Gnathia pilosus*). Some parasites found in the inner surface of the operculum.

Crustaceans can affect fish, they attached to gill filaments causing pathological lesions such as necrosis in branchial epithelial tissue, desquamation, increase of mucus secretion, erosion, occlusion of the branchial circulation and destruction due to hypertrophy and the pressure of feeding (*Kabata 1970, Manera and Dezfuli 2003, Toksen et al. 2008, Banu and Zafer 2012 and Eissa et al. 2012*). 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 and then death.

Parasitic copepods have worldwide distribution and are economically important parasites in marine aquaculture (**Kabata, 1970**). Disease outbreaks and mortalities caused by *L. kroyeri* are frequently observed in seabass culture and economic losses occur due to growth reduction, reduced feed conversion, loss of product value, treatment costs and mortality (**Manera and Dezfuli, 2003**). Based on the parasitological examination, the isolated crustaceans were identified as *Lernanthropus kroyeri* van Beneden, 1851, *Caligus minimus*, (Otto, 1821) and the 3rd stage of praniza larva *Gnathia pilosus* according to the description of **Ozak (2006)**, **Henry et al. (2009)**, **Ercument et al. (2011)**, **Antonelli et al. (2012)**, **Bayoumy et al. (2013)** and **Banu et al. (2014)**.

*Lernanthropus kroyeri* isolated from gills of *D. labrax*, nearly agree with the findings given by **Badawy (2001)**, **Akmrza (2003)**, **Korun and Tepecik (2005)**, **Henry et al. (2009)** and **Eissa et al. (2012)** who isolated the same genus from the same site and host. Also, it is in agreement with **Manera and Dezfuli (2003)**, **Toksen et al. (2008)**, **Henry et al. (2009)** and **Antonelli et al. (2012)** who obtained *L. kroyeri* from the same site and host and disagree with **Toksen et al. (2012)** who obtained the same genus from white

grouper *E. aeneus* and with **Roubal (1986)**, **Luque et al. (1989)** who obtained the same genus from *Seriolella violacea*, *Paralonchuri peruanus*, *Anisotremus scapularis* and *Acanthopagrus australis* respectively. The site of infestation was in agreement with that mentioned by **İsmet Özell et al. (2004)**.

*Lernanthropus kroyeri* van Beneden, 1851 has been recorded from many localities along the coast of Europe, from the Adriatic Sea to the southern North Sea. *D. labrax* was the only host in all these waters (**Kabata, 1979**). *Caligus minimus* was isolated from the gills and inner surface of the operculum of *D. labrax*, this agrees with **Paperna (1980)** and **Banu et al. (2014)** who isolated the same species from the same host and site and **Tansel and Fatih (2012)** who isolated the same species from brown wrasse (*Labrus merula*) and with **Cressey (1991)** and **Badawy (1994)** who isolated the same genus from gills of *Caranx sem* and with **Kabata (1988)** who isolated it from skin and with **Oldewage (1990)** who examined the buccal cavity of *Arthron hipidus* and collected the female parasite and **Maran et al. (2009)** who isolated the same genus from body surface and gill cavities of marine fishes in Malizia.

The 3rd stage of praniza larva *Gnathia pilosus* was isolated from mouth cavity of white grouper *Epinephelus aeneus* and this was in agreement with **Ercument (2007)**

who isolated the same genus from the epithelium of the buccal cavity of Dusky grouper (*Epinephelus marginatus*). But differed from that obtained by **Ercument et al. (2011)** who extracted the same genus from the internal side of the gill arch and epithelium of the buccal cavity of the goldblotch grouper (*Epinephelus costae*), **Bayoumy et al. (2013)** who isolated the same genus from pectoral fins, gills and mouth cavity especially palate and tongue of *Epinephelus tauvina*.

Regarding crustacean infestations, the total prevalence was 18%. This result is nearly similar to that recorded by **Maather El-Lamie (2007)** as it was 15.67% and **Vagianou et al. (2004)** as 13.6% while it is much higher than that obtained by **Engi El-Raziky (2009)** as it was 7% and **Badawy (2001)** as it was 2.25%. This difference may be attributed to the time, age, number of fish and locality from which fish samples obtained.

Regarding crustacean infestations (Copepodiasis) among *D. Labrax*, the total prevalence was 36%. This result is in agreement with **Manera and Dezfuli (2003)** who detected *L. kroyeri* (35%) among *D. labrax*. While it was higher than that obtained by **Banu et al. (2014)** who found *C. minimus* (29.8%) among *D. labrax* and that obtained by **Badawy (2001)**, **Abd El-Aal (2003)**, **Vagianou et al. (2004)** and **Engi El-Raziky (2009)** as it was 2.25, 10.43, 13.6 and 7% respectively. This difference may

be attributed to the locality from which fish samples obtained. On the other hand, our result is lower than that obtained by **Eissa et al. (2012)** as it was 47%.

Also, the prevalence was in disagreement with that recorded by **Badawy (2001)** who found no infestation in *M. labrax*, **Eissa et al. (2012)** who detected *Caligus carangis* (29%) and *Lernanthropus pisciana* (18%) among *Morone labrax*, **Elgendy et al. (2015)** who detected *Caligus elongates* (92.3%) in *D. labrax*.

Regarding Crustacean infestations among white grouper (*E. aeneus*), the total prevalence was 18%. This result was lower than that recorded by **Ercument et al. (2011)** who isolated gnathiid from goldblotch grouper (*Epinephelus costae*) with prevalence of 28.12% and **Bayoumy et al. (2013)** who isolate pranzia larva of *Gnathia pantherina* from greasy grouper (*Epinephelus tauvina*) with prevalence of (58.33%).

Regarding the seasonal prevalence of crustacean infestation, it was the highest in autumn 28% followed by spring 24% then summer 16% and the lowest was winter 4%. This sequence agrees with **Maather El-Lamie (2007)** who found that the highest infestation was recorded in autumn 76% and the lowest was in summer 16%. However, **Mai Mohamed (2013)** found the lowest infestation was recorded in winter 1.33%, **Badawy (2001)** who found that the infestation rate was

declined in winter, **Doaa Faisal (2008)** found that the lowest infestation was recorded in winter season. However, disagreed with **Ragias *et al.* (2004)** who found that peak of intensity in winter, **Engi El-Raziky (2009)** who found that the highest infestation was recorded in winter 16%, while the lowest one was in summer 0%. This difference may be due to the geographical distribution of the hosts and parasites.

Regarding the seasonal prevalence of crustacean infestation among *D. labrax*, it was shown that the highest in autumn 84%, followed by summer 48% then spring 12% and winter 0%. This disagrees with the results recorded by **Badawy (1994)** who found that the highest infestation rate was recorded in summer season and the lowest was recorded in the winter season. **Eissa *et al.* (2012)** who found *Lernanthropus pscianae* and *Caligus carangis* among *Morone labrax* and recorded that the highest infestation was in summer season (76%) and winter was the lowest season (16%) for infestation and **Banu *et al.* (2014)** who recorded that *C. minimus* in *D. labrax* as the peak was the highest in spring 45% and the lowest in summer.

Regarding the seasonal prevalence of crustacean infestation in *E. aeneus*, it was found that the peak was the highest in spring (60%), followed by winter (12%) and the lowest was recorded in autumn and summer (0%). This disagrees with

the results obtained by **Ercument *et al.* (2005)** who recorded that the prevalence of infestation gradually increased during the spring and reached peaks during the summer. They added that the prevalence of infestation was not observed in winter season.

Regarding prevalence of crustacean infestation in relation to length. It has been found that, there was positive correlation between crustacean infestation and fish length. Fishes of high body length are more exposure to external parasites than small length. These results were in agreement with **Guegan *et al.* (1992)** and **Sasel *et al.* (1997)**.

*D. labrax* and *Epinephelus aeneus* showed no clear correlation between prevalence of crustacean infestation and body weight of fish. These may attributed to the unsimilar number of the examined fishes at each weight.

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