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Original Article

Studies on the Prevailing Parasitic Diseases in Some Marine Fishes

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Abstract:

This study has been applied on 200 marine fish (100 *Dicentrarchus labrax* and 100 *Scomberomorus commerson*) that randomly collected from Ismailia Provinces. No pathognomonic clinical abnormalities were recorded, however some infested fish showed hemorrhagic areas on gill cover, abdomen and on the bases of fins with presence of one or more isopoda with excessive mucus secretions. The postmortem findings were Marbling of the gills with excessive mucus secretion, sticking of the gill tips and greyish coloration. In some cases, liver was pale with peticheal haemorrhage, Stomach and intestine showed congestion, enlargement, thickening and inflammation of their walls. The total prevalence of parasitic infestation among examined fishes was 59%. The highest percentage was in *Scomberomorous commerson* (88%) followed by *D. labrax* (29%). The identified parasites were monogenetic trematodes (55%), Acanthocephalan (1.5%),) and Nematodes (3% (.The total seasonal prevalence of the detected parasites was the highest in summer (66%) and the lowest in autumn (48%). The relationship between length and parasitic prevalence was recorded. Molecular detection of Diplectanidae family using conventional PCR technique was evaluated using a DNA mixture prepared from the target pathogens. It was conducted using two universal primers for Trematode (ITS-2), which yielded amplification products of 539 bp. As expected, a PCR product of predicted size (539 bp) was generated in all examined samples and was finally identified as Diplectanum species. Histopathological changes of the naturally infestedfish with various parasites were recorded.

Key words: Marine fishes, Parasites, signs, lesions, histopathology.

Introduction:

Fish is one of the most valuable sources of animal protein. Worldwide, people obtain great part of their animal protein from fish and shell fish. The need for fish as a source of protein grows as the human population grows. Parasitic invasions represent the common known infectious diseases affecting fish **(Eissa *et al*., 2012).** In addition to the economic losses to farmers due to parasitic diseases, some parasites are of zoonotic importance. Eating raw or improperly cooked or processed fish is the main source of these infections to human that has been reported from various geographical regions **(Park *et al*., 2009)**. Sea bass (*Dicentrarchus labrax*) and darak *(Scomberomorus commerson)* are two of the chief marine fish species widely reared in the Mediterranean area. In Egypt, Sea bass fish products have reached to 19.027 tons **(Macfadyen *et al*., 2012).** The steady rise in the production of fish resulted in severe pathological impediments in all countries including Egypt where intensive aquaculture is accomplished. Parasitic

diseases affecting marine fishes are numerous and they cause high losses in marine culture sector in Egypt especially in sea bass and darak. (**Khalil *et al*., 2014)**. Helminthes are among the most important parasites, they include nematodes, trematodes, cestodes and acanthocephalans which affecting both wild and cultured marine fishes **(Hussen *et al*., 2012).** These parasites cause some diseases that closely linked to environmental deterioration and stress. Crustacean diseases are considered an important limiting factor in the development of intensified marine fish culture **(Osman *et al*., 2014)**.Among marine fish parasites, approximately 25% are crustaceans, mainly signified by copepod, branchiura and isopoda **(Eiras *et al*., 2000) .**They have a great economic importance as agents of disease in wild and aquacultured fish populations **(Rohde, 2005)**. They affect growth fecundity andsurvival of wild hosts **(Bayoumy and Hanady Baghdadi, 2013).**

In systems of thorough culture, complications of infestation triggered by protozoan and metazoan parasites are fairly frequent. Metazoan parasites lead to gill affections, eyes and internal organs damages, starvation, irritation of the swim bladder, and inhibited oxygen interchangeamong gill lamella **(Wandersone*t al*., 2012).** This study was aimed to investigate different parasitic infestations in some marine fishes at Ismailia Province, Egypt, represented as sea bass and darak.

Materials and Methods:

Fishes:

A total of 200 marine fishes of 2 species represented as "100 *Dicentrarchus labrax* and 100 *Scomberomoru scommerson*" of different body weights and lengths were collected in different seasons from Ismailia Province. They were collected between September 2015to the end of August 2016. They were obtained by the aid of fishermen and fishing gears, then transported immediately to the laboratory of fish Disease and Management, Faculty of vet. Medicine, Suez Canal University alive in polyethylene bags containing 1/3 of its volume water where the remaining volume was filled with air.

A. Identification of marine fish species:

It was adopted according to **Randall (1983).**

B. Clinical examination:

Clinical examination was made on the live or freshly dead fishfor detection of any clinical abnormalities according to **Amlacker (1970)**. The postmortem examination was performed on all fishes according to **Lucky (1977)**.

C. Parasitological examination:

Fish specimens were examined macroscopically and microscopically for external and internal parasites as soon as possible after they were sacrificed.

D. Permanent slides smear preparations and staining:

Monogenetic trematodes: The detected worms (separated or within gill tissue) were fixed in formalin 3% then a drop of glycerin alcohol (1:4), dehydrated in ascending grades of ethyl alcohol (3, 50, 70, 80, 90, 100%), cleared with clove oil, then xylene to remove the oil (each step take 15-30 minutes) and mounted in Canada balsam then left to dry in horizontal position in hot air oven **(NegmEldin and Saleh, 1995).**

Nematodes: The collected nematodes from stomach and intestine were washed in saline, then relaxed and fixed in hot alcohol-glycerin 5% until all alcohol evaporated and the specimen remained in nearly absolute glycerin and processed according to **Meyer and Olsen (1992).**

Acanthocephala: The worms were compressed in between 2 slides and fixed in 4% formalin in and cleared, fixed and mounted as digeneans or cleared with glycerin as nematodes.

E. Identification of the isolated parasites:

- Monogenetic trematodes were identified according to **Yamaguti (1934)**, **Yamaguti (1958) and Pamplona-Basilio*et al*., (2011)**.
- Nematodes were identified according to **Ramachandran (1973)**.
- Acanthocephala were identified according to **Monteiro *et al*. (2006)**.

F. Detection of Diplectanid monogenea using PCR:

Samples of DNA were obtained from larvae of infected fishes following clearance with SDS 1%. DNA was extracted with QIA amp DNA mini kit(GIAGEN, USA) Ref. No (51304) according to manufacturer's instructions. To ensure a good-quality input of DNA, the isolated DNAs were investigated for proper concentration and integrity using agarose running gel assay. To ensure that the isolates were belong to Trematode, two sets of universal primers representing variable regions on **ITS2** gene were selected according **Arya *et al*. (2016)**, and gave amplification bands of 539bp table (1)

Table(1) Oligonucleotide probs (primers) Biobasic, Canda

Targ-et gene	Prim-er	sequence, 5′→ 3′	DNA amplified(bp)
ITS2	F	GGTACCGGTGGATCACTCGGCTCGTG	539
	R	GGGATCCTGGTTAGTTTCTTTTCCTCCGC	

All PCR amplifications were performed using commercial Emerald Amp GT PCR MasterMix (Takara) Ref. No (RR310A). In all PCR experiments, DNA from pure cultures of Trematodewas included as a positive control, whereas molecular biology water was used as a negative control. Amplified products were then detected by horizontal 1.5 % (w/v) agarose gel electrophoresis for 30 min at 1-5 volts/cm. After gel separation, the amplified products were visualized using 20 µL of DNA gel stain (Sigma) under UV transilluminator, photographed using a polaroid MP-4 camera and computer digitized (Gel Doc 100, Bio-Rad). A 100 bp ladder Gel Pilot (QIAGEN, **USA)** was used as a molecular mass marker

G. Histopathological examination:

Tissue specimens from the infested organs were taken, fixed immediately in 10% neutral buffered formaline, dehydrated in ascending grades of alcohols, cleared in xylene then blocked in paraffine wax, sectioned at 5-7 microns and stained with H&E according to **Carleton (1976).**

Results:

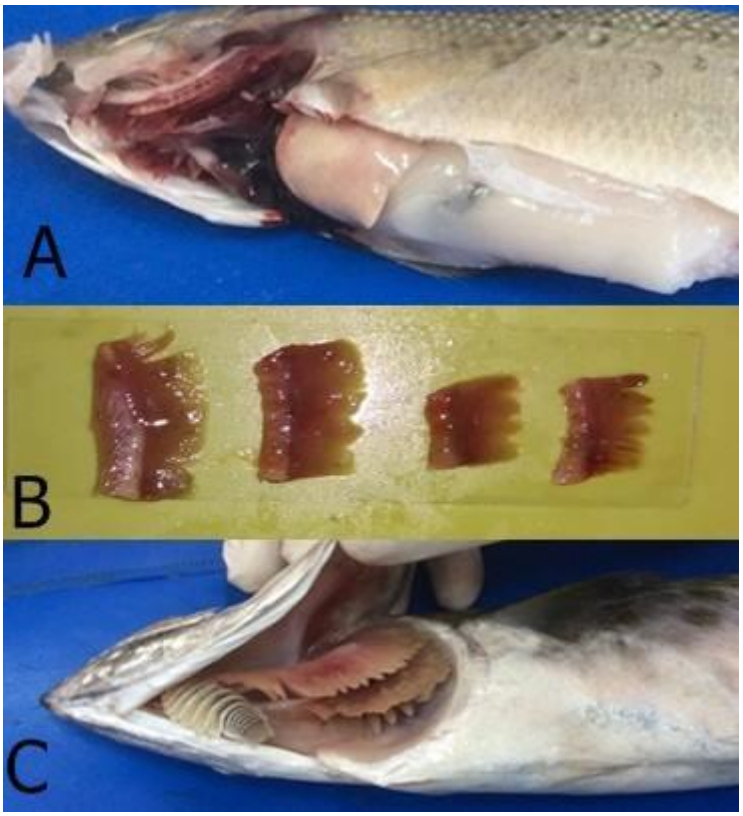
Clinical examination of naturally infested fishes:

The clinical signs in the naturally infested fishes (*D. labrax* and *S. commerson)* showed no pathognomonic

clinical abnormalities. Some infested *D.labrax* showed hemorrhagic areas on gill cover, abdomen and on the bases of fins**,** abdominal distension and somewhat emaciation, with presence of one or more isopoda with excessive mucous secretions. The infested *S. commerson* showed no external abnormalities except bulging of opercula with presence of one or more isopoda in both sides that may cause in some cases absence of large part of gill cover **(Plate 1).**

Postmortem examination:

Gills showed a marbling (mosaic) appearance with excessive mucus secretion. Gill tips were sticking with grayish coloration in *D.labrax* . In *S. commerson* many cases there was destruction of gill filaments and some were infested with monogenetic trematodes. Internal examination as spleen and kidneys showed no abnormality. On the other hand, liver was pale in some examined fishes with petechealhemorrhage in other some cases. Stomach and intestine showed congestion, enlargement, thickening and inflammation of their walls in some examined *D.labrax*. (**Pate 1)**



Plat (1): (A): *D. labrax* showing pale liver (B): Gills of D. labrax with mosaic appearance, sticking of the gills and grayish coloration (C): *S. commerson* showing isopoda attached to the gills.

Parasitological examination:

Fish specimens were examined parasitologically (macroscopically and microscopically). Identification of the parasites was carried out according to its morphometric measurements as follows:

I) Gill monogeneans:

1- **Diplectanum sp. Yamaguti 1963 (Plate 2).**

2- **Microcotyle sp. Yamaguti 1963 (Plate 2).**

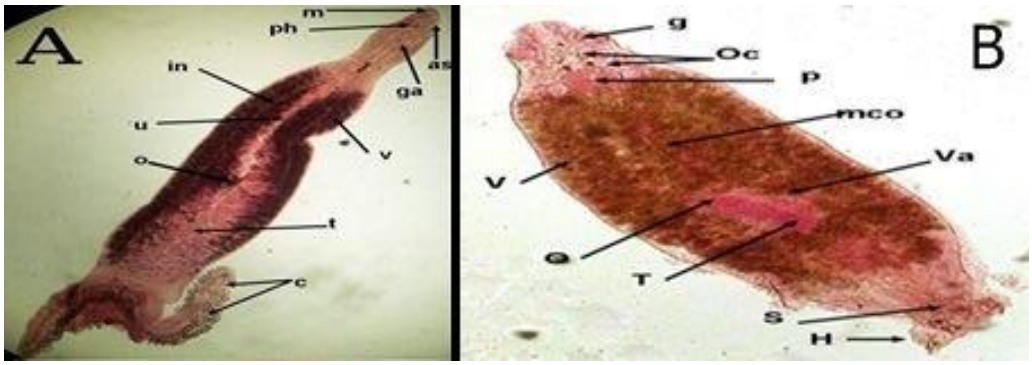


Plate (2): (A).Microcotyle sp.: m= mouth; as= anterior sucker; ph= pharynx; ga= genital atrium; in= intestine; u= uterus; v= vetillaria; o= ovary; t= testes; c= clamps.**. (B): Diplectanum sp.: g:** glands of head; Oc: Oculi; P:Pharynx; Mco: male copulatory organ; Va: Vagina; O: Ovary; T: Testis; V: Vetillaria; H: Haptor; S: Squamodiscs

II) **Intestinal nematodeasis:**

***Hysterothylaciumaduncum*Rudolphi 1802(Plate 3)**

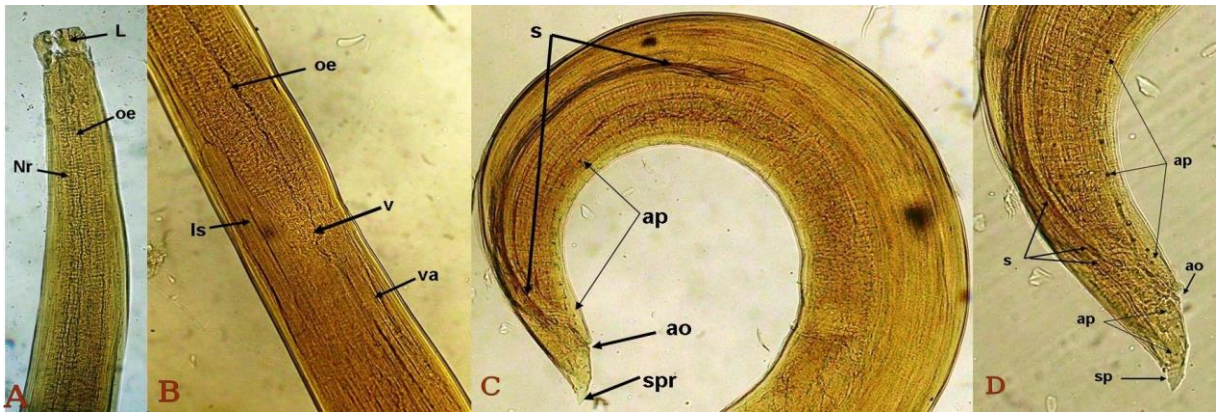


Plate (3): A. Anterior part of adult *Hysterothylaciumaduncum*: L= lips; oe= oesophagus; Nr= Nerve ring. **B. Ventriculus part ofadult*Hysterothylaciumaduncum*:**oe= oesophagus; ls= Intestinal caecum; v= ventriculus; va= ventricular appendix. **C. Posterior part of adult *Hysterothylaciumaduncum*:** s= two spicules; ap= anal papillae; ao= anal opening; spr= spinose process. **D. Posterior end of adult *Hysterothylaciumaduncum*:** s= two spicules; ap= anal papillae; ao= anal opening; spr= spinose process

III) Intestinal Acanthocephalans*:*

1- Rhadinorhynchus sp. Margolis and Kabata1989 (Plate 4)

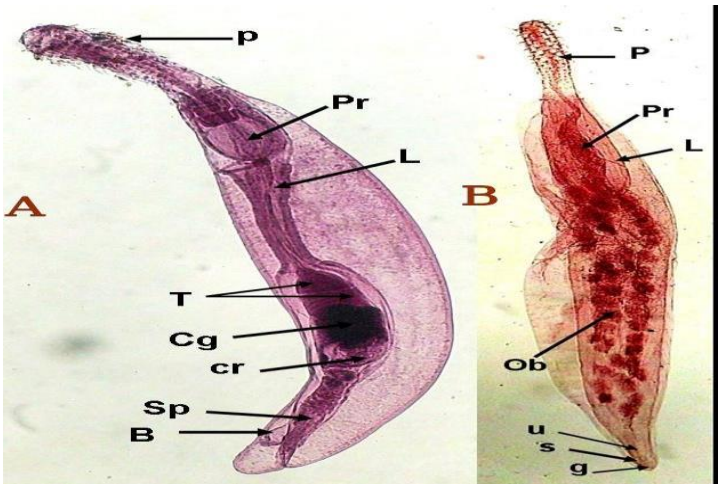


Plate (4): A. **Adult male ofRhadinorhynchussp.:** P=Proboscis; Pr= Proboscisreceptacle; L=lemnisci; T= Testes; Cg= Cement gland; Cementreservoir; Sp= saefftigen spouch; B= bursa. **B**. **AdultfemaleRhadinorhynchussp.:**P=Proboscis; Pr= Proboscisreceptacle; L=lemnisci; ob= Ovarianballs; u= uterus; s= sphincter; g= genital gland.

Prevalence and seasonal variations of parasitic infestation among different examined fish species:

Table (2&3) shows the total and seasonalprevalence of parasitic infestationamong the examined marine fishes.

Table (2): Total & seasonal prevalence of parasitic infestation among *D. labrax* fish.

Parasitic sp.	***Autumn***		***Winter***		***Spring***		***Summer***		***Total (n=100)***	
Season (n=25)	***No. inf.***	***%***	***No. inf.***	***%***	***No. inf.***	***%***	***No. inf.***	***%***	***No. inf.***	***%***
Monogeneas	**2**	**8**	**9**	**36**	**8**	**32**	**3**	**12**	**22**	**22**
Nematodes	**2**	**8**	**0**	**0**	**1**	**4**	**0**	**0**	**3**	**3**
Acanthocephalans	**0**	**0**	**0**	**0**	**1**	**4**	**2**	**8**	**3**	**3**

N = number of examined fish samples.

Table (3): Total & seasonal prevalence of parasitic infestation among *S. commerson* fish.

Parasitic sp.	***Autumn***	***Winter***	***Spring***	***Summer***	***Total (n=100)***

Season (n=25)	*No. inf.*	*%*	*No. inf.*	*%*	*No. inf.*	*%*	*No. inf.*	*%*	*No. inf.*	*%*
Monogeneas	**17**	**68**	**23**	**92**	**23**	**92**	**24**	**96**	**87**	**87**

N = number of examined fish samples.

Molecular detection of Diplectanidae family using conventional PCR technique:

The specificity of the method was evaluated using a DNA mixture prepared from the target pathogens. It was conducted using two universal primers for Trematode (ITS-2), which yielded amplification products of 539 bp. As expected, a PCR product of predicted size (539 bp) was generated in all examined samples and was finally identified as Diplectanum species **(Photo 1).**

L	Pos	2	1	Neg

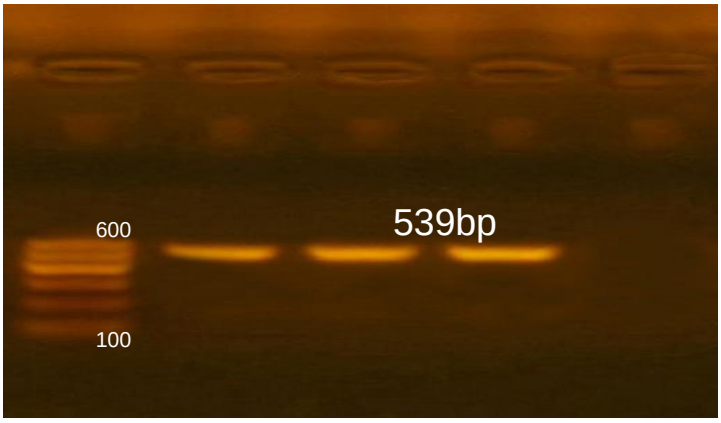


Photo (1): A representative gel displaying the ssrDNA analysis of ITS-2 region from individual adult specimens of Diplectanum sp. Lane 2,1 at 539 bp. Lane L represent the 100 bp DNA ladder as marker (bp).

Histopathological examination of the infested fishes:

Microscopical examination of the skin of the naturally infested *D.labrax* with isopoda marked vacuolar degeneration in epidermal cells was evident, while the dermis exhibited edema and some leukocytes. While in *S. commerson* the microscopical examination of the skin showed marked vacuolar degeneration in epidermal cells was evident with focal epidermal ulcer. On the other hand musculture of *D.labrax* infested with isopoda revealed that the under laying muscle showed intermuscular edema with focal hyaline degeneration and Zincker'snecrosise. The necrotic muscles were infiltrated with mononuclear cells

and melanomacrophages**.** The microscopical examination of the gills of the naturally infested *D.labrax* with monogenetic trematode revealed massive destruction in both primary and secondary lamellae. Mononuclear cells infiltrations were evident in the gill lamellae**.** In the intestine of *D.L* the microscopical examination showedalternative mucinous degeneration to coaguative necrosis in the epithelial lining. The lamina propria and submucosa were infiltrated with mononuclear cells. **(Plate 5)**

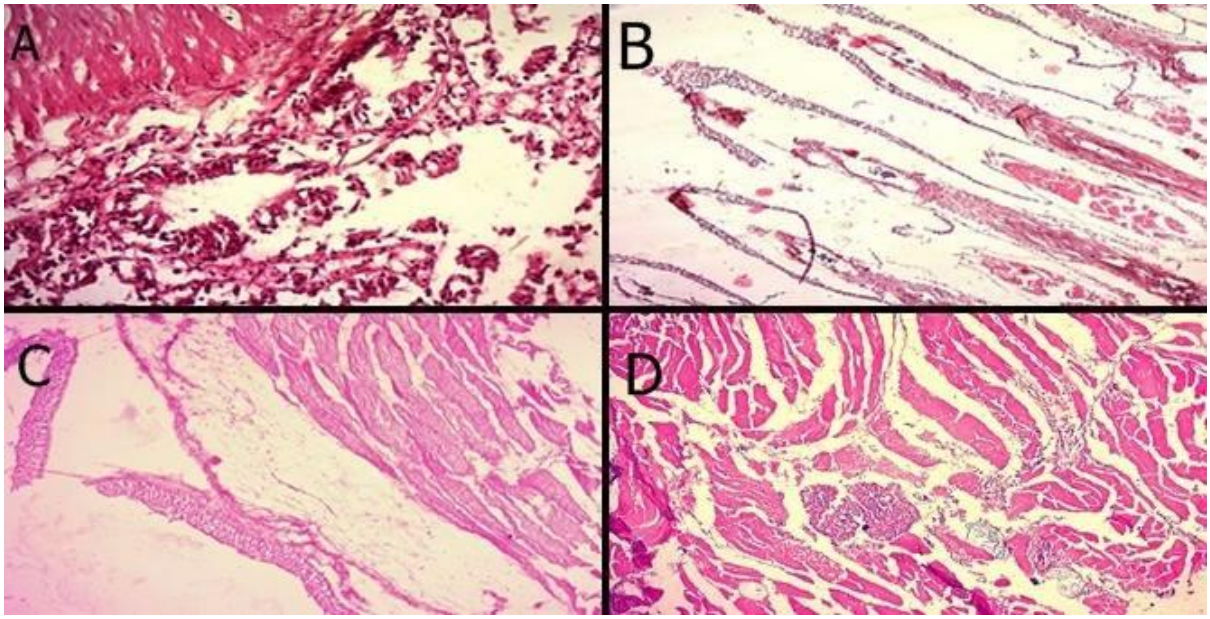


Plate (5): (A) Intestine of *D.labrax* infected bynematode showing massive necrosis in the granular epithelium with mononuclear cell infiltration in both lamina propria and submucosa. . H&E stain ×400. **(B)** Gills of*D.labrax* with monogenetic trematode showing marked sloughing in the primary and secondary lamellae with mononuclear cells infiltration. H&E stain ×250. **(C)**: skin of *S.commersons* infected byisopoda showing epidermal ulcer with edema and mononuclear cell infiltration in the cells. H&E stain ×250. **(D)** Musculture of *D.labrax* infected by isopoda Showing intermuscular edema hyaline degeneration and Zenicker's necrosis. H&E stain ×250.

Discussion:

Suez Canal area is important part of Egypt either for its economic importance as source of income or as source of fish production in many governorates. The main purpose of the current work is the determination of prevailing parasitic diseases affecting some marine fishes (*D.labrax* and *S.commerson*) and their impact on the fish health as well as the study of seasonal prevalence of these affections throughout the different seasons. The main clinical signs observed in infested fish as mentioned before are in agreement with those reported by **Maather El-lamie (2007), Doaa Faisal (2008) and Khalil *et al*. (2014).).** Postmortem examination in some infested fish with internal parasitic infestation (nematode) showed no abnormality in spleen and kidney. On the other hand, liver was pale in some examined *S. commerson* and pale with peticealhaemorrhage in other some cases. Stomach and intestine showed congestion, enlargement, thickening and

inflammation of their walls in some examined *D. labrax*. This was agree with **Bassiony (2002), Eissa (2002), Ibtesam Eissa (2004) and Heba Abdel – Moula (2005).**

Regarding the identification of Diplectanid Monogenean parasite using PCR, the nomenclature and taxonomy of Diplectanide species has since become controversial and confusing **Beverley-Burton and Suriano (1981) and Wu *et al*., (2005**). Therefore, the molecular methods established here in provide useful tools for future investigations of specimens from a wide range of fish species and geographical origins. In this study, molecular identification of Diplectanum spp. using PCR analysis of the ssrDNA (ITS2) (size 539bp) are a common molecular identification. The ITS-2 amplicons of size (□ 539bp) were agree with **Li *et al*. (2005)** who found it (from 180bp to 780bp).

Although morphometric keys are available for the identification of adult specimens of Diplectanid sp., no such keys are available for the specific identification of larval stages. Hence, the PCR analysis approaches established provide useful tools for the accurate identification of species of Diplectanum (irrespective of developmental stage), providing a foundation for investigating their ecology (e.g., host preference and host-parasite relationships) and population genetic structure and for the control of disease they cause

The present study indicates that the prevalence of monogenean parasites was the highest in *S. commerson* (87%) followed by *D. labrax* (22%). The differences between fish species may be due to the type fish itself, host pathology, post spawning migration of the host and host immunity response. This result differed from that obtained by **Abdel Aal *et al.* (2001)** as it gave results for each parasite alone and not a total prevalence and may be due to the differences of locality from where the samples were collected.

Regarding the acanthocephalan infestation, the highest prevalence was recorded in *D.labrax*3%, on the other hand *S. commerson* were free from Acanthocephalan infestation.

The seasonal prevalence of monogenetic trematodes in *D. labrax* shows the highest rate in winter (36%) followed by spring (32%) and summer (12%) then autumn (8%). In *S. commerson*, the prevalence was the same (92%) in winter and spring while in summer and autumn it was (96%) and (68%) respectively. This disagree with **Bayoumy (2003)** whofound that the highest incidence was in summer (60.8%) and the lowest incidence was in winter (26.2%), **Mohamed *et al*. (2015)** who reported that the highest season of infection was in summer with prevalence of 39.16%, followed by autumn 27.22%, spring 21.11% and the lowest infestation rate was recorded in winter, 12.50% and **Roumbedakis *et al.* (2012)** who recorded a 100% prevalence all over the year as they found that monogeneans infestation is temperature dependent while this is nearly agree with **El-Etreby *et al.* (1993)** who found that seasonal infestation not follow a regular behavior in the examined fishes and with **Rawson and Rogers(1972)** who found monogeneans parasites showing peaks of abundance during cold seasons. This may be due to the lower levels of specific antibodies produced by the host during cold seasons **Cloutman (1978)**. Also, life span of the free swimming monogeneans

larvae is temperature independent **Paperna (1980)**. Regarding the seasonal prevalence of Nematode and Acanthocephalan infestation was only recoded in *D. Labrax.* For nematode the prevalence was 8% in autumn, 0% in winter, 4% in spring and 0% in summer. This result of nematode infestation disagrees with **Roumbedakis*et al.* (2012)** and **SamahEl shafey (2016)** in that the peak of infestation was in spring. For acanthocephala it was observed with prevalence of 0% in autumn and winter, 4% in spring and 8% in summerthis disagree with **Santos *et al*. (2005)** who found the highest prevalence occurred in October and February (100%) followed by June and July (90%) then August 2000 and September (80%) then December (75%) then May (67%) then April (20%) and finally August 2001 (10%) and **Maather El-lamie (2007)** who found the highest peak of Acanthocephalan infestation was observed in winter season only (20%). In the present study, histopathological changes in the skin of infested fishes with isopoda revealed marked vacuolar degeneration in epidermal cells, while the derms exhibited edema and some leukocytes with focal epidermal ulcer. On the other hand muscles of *D.labrax* infested with isopoda revealed that the under laying muscle showed intermuscularedema with focal hyring degeneration and zincker'snecrosise. The necrotic muscle were infiltrated with mononuclear cells and milanomacrophages**.** These results were in agreement with **Purivirojkul(2012), Jerônimo*et al.* (2014) and Khalil *et al.* (2014)**

The histopathological changes due to monogenean infestations of gills of the examined fishes were presence of the parasite on the surface of the gill filaments in the form of basophilic part, massive destruction in both primary and secoundry lamellae. severe hyperplasia, severe congestion of branchial blood vessels, mononuclear cell infiltration and vacuolation of the epithelial lining of the secondary lamellae. This result is in agreement with that obtained by **Mahi Ghobashy (2000), Maather El-Lamie (2007) and Hossain *et al*. (2007).** Concerning the histopathological changes in the intestine of *D.L* the microscopical examination showes alternative mucinous degeneration to coaguative necrosis in the epithelial lining. The lamina propria and submucosa were infiltrated with mononuclear cells. These results were in agreement with that obtained by **Martins *et al.* (2001), Heba Abdel-Moula (2005)** and **Maather El-lamie (2007)*.***

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الملخص العربى

دراسات عن الامراض الطفيليه السائده في بعض الاسماك البحريه

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أجريت هذه الدراسة و تم تجميع ٢٠٠ سمكة بحرية بواقع ١٠٠ من القاروص،١٠٠من الدراك عشوائيا من المياه المالحة لمدينه الإسماعيلية فى المواسم المختلفة و قد تميزت الأسماك بالأحجام و الأوزان المختلفة.أسفر الفحص الإكلينيكى للإسماك المصابة بالطفيليات المختلفة عن عدم وجود علامة مرضية مميزة. بعض الأسماك المصابة أظهرت تضخما للغطاء الخيشومى، نزيف ، خدوش ، انتفاخ البطن و الهزال كما أظهر فحص تلك الأسماك بعد نفوقها عن احتقان مع شحوب فى الخياشيم مع زيادة الإفراز المخاطى و التصاق حروف الخياشيم ببعضها مع تلونها باللون الرمادى. فى بعض الحالات المصابة أظهر الكبد شحوبا مع وجود نقط نزفية و أظهرت المعدة والأمعاء احتقانا و تضخما مع التهابها.

كانت النسبة الكلية للإصابه ٥٩%. سجلت أسماك الدراك أعلى نسبة إصابه (٨٨%) وأتبعت بأسماك القاروص (٢٩%) وكانت الطفيليات المصنفة هىديدان مفلطحة أحادية العائل و التى عزلت بنسبة ٥٥%, ديدان رأس شوكية والتى عزلت بنسبة١.٥ %, , الديدان الإسطوانية والتى عزلت من اسماك القاروص بنسبه ٢% .سجلت النسبة الكلية الموسمية للطفيليات المعزولة اعلى نسبة اصابة فىالصيف (٦٦%) وأقل نسبة إصابة فى الخريف (٤٨%). تم تصنيف احد الديدان المفلطحه وحيده العائل من عائله دبليكتانيوم باستخدام ال PCRوسجلت ظهورقاعده ثنائيه النيتروجين فى خلايا رقم ٢,١ عند ٥٣٩bp.وقد سجلت الصورة الهستوباثولوجية للاعضاء المصابة.