

## Seasonal Survey on Common Parasitic Diseases in *Dicentrarchus labrax*, *Dicentrarchus punctatus* and *Sparus aurata* Collected From Lake Timsah, Egypt

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### ABSTRACT

This study aimed to identify parasitic species infesting marine fish collected from Lake Timsah across different seasons. A total of 480 marine fish of varying species, weights, and lengths (160 *Dicentrarchus labrax*, 160 *Dicentrarchus punctatus*, and 160 *Sparus aurata*) were randomly collected seasonally from September 2020 to August 2021. Most examined fish showed no pathognomonic clinical abnormalities and appeared normal. However, some *D. labrax* exhibited hemorrhages on the mouth, operculum, around the eyes, and at the bases of their fins, along with abdominal distension and emaciation. Infested *D. punctatus* showed excessive mucus and a mosaic appearance of the gills. Infested *S. aurata* displayed no external clinical abnormalities, except for some hemorrhagic lesions and slight abdominal distension. The total prevalence of parasitic infestation among the examined fish was 57.91%. The highest infestation rate was observed in *D. punctatus* (74.38%), followed by *D. labrax* (71.25%) and *S. aurata* (28.13%). The isolated parasites included nematodes (*Hysterothylacium aduncum*, *Anisakis simplex* larvae, and *Contracaecum* spp. larvae), digeneans (*Erilepturus hamate* and *Tangiopsis chinensis*), crustacean parasites (*Lernanathropus kroyeri*, *Caligus minimus*), and isopods (*Livoneca redmanii*). The total parasitic infestation rate varied significantly across different seasons ( $P < 0.001$ ), with the highest rate in winter (81.6%), followed by spring (70.8%), autumn (55%), and summer (24%). Several factors, such as season, fish species, and differences in body weight and length, affect the infestation rates.

### INTRODUCTION

Lake Timsah, the main site of the study, is not only one of the well-known wetlands in the Suez Canal region and a main tourist attraction, it is one of the main sites in Egypt where vast numbers of migratory birds are passing through, especially during winter on their way from Europe to Africa (Varó *et al.*, 2002). The lake is the backbone of a tourism industry that attracts a large number of holiday visitors. In addition to attracting visitors and supporting the tourism and fishing industries, Lake Timsah is a source of fish, crustaceans, and shellfish, which employ local citizens and contribute significantly to the district's revenue (Senthilkumar *et al.*, 2001; Ahmed, 2005).

Fish and seafood are considered important sources of high quality protein, minerals and essential polyunsaturated fatty acids (Guerin *et al.*, 2011). Marine fishes are preferred over freshwater fishes due to their abundance in trace elements (Eissa *et al.*, 2015), subsequently, these species have been the principal species used in the Egyptian marine aquaculture starting in the 1990s. The production of these two species peaked at 18,900 tonnes in 2000 (DE *et al.*, 2010). The European seabass *Dicentrarchus labrax* (Linnaeus, 1758) is one of the most valuable commercial fish not only in the Mediterranean countries but also worldwide (Enes *et al.*, 2012; GSA, 2023). However, the astonishing increase in the biomass of the mariculture facilities could lead to several risks including stress factors, depressed immune system, pathological problems, and subsequent negative impacts on the water environment (Sapkota *et al.*, 2008; Aly, 2013; Essa *et al.*, 2018). Parasitic infections are considered great problems facing aquaculture, which may cause morbidities, mortalities, and thus economic losses. Under natural conditions, they comprise the parasite fauna of total biomass, but they could become pathogens to their hosts, invading various external and internal tissues, reducing the fish quality, and harming humans (Tansel & Fatih, 2012). Among the common parasitic diseases reported in various fish species are those affecting crustaceans, including isopods and copepods (Manera & Dezfuli, 2003). Moreover, other parasitic species such as trematodes, cestodes, acanthocephalans, and nematodes are considered major causes of parasitic diseases in aquatic ecosystems. They are extremely important and can cause significant tissue damage (Noga, 2010; Aly *et al.*, 2020). Fish parasites invade fish for shelter and nourishment. In the end, the parasite triumphs against the fish. These parasites investigate the possibility of generating a weakened immune system in the host, increasing their vulnerability to subsequent infections, resulting in nutritional devaluation of fish (Onyedineke *et al.*, 2009) and significant losses in Egypt's marine culture sector (Khalil *et al.*, 2014). Generally, parasites that cause damage and even death in marine fish use them as either final or intermediate hosts (Abdel-Mawla & El-Ekiaby, 2012). Additionally, endo-parasitic metazoans may use marine fish as a paratenic host (Navarro *et al.*, 2019). Ecto and endo parasites are the two types of helminth parasites, depending on whether they dwell on the surface or inside their hosts. Internal parasitic disorders that alter normal physiology of fish and have detrimental effects on the operations of the affected organs have lowered fish productivity. Endoparasites absorb large amounts of nutritional substances from the host through their body surfaces (Banerjee *et al.*, 2017) resulting in emaciation and anemia. Moreover, they cause enlargement and congestion of the internal organs (Eissa, 2004). Additionally, they pose a health risk to humans and other invertebrates who eat diseased fish. Ectoparasitic diseases also result in significant economic losses. They open dermal wounds which allow secondary infections to develop, resulting in high mortality rates (Noga, 2010). The current study aimed to enhance our understanding of the parasitic fauna present in Lake Timsah among certain marine fish species.

## **MATERIALS AND METHODS**

### **1. Sample collection**

A total of 480 marine fishes of 3 species represented as "160 *Dicentrarchus labrax* and 160 *Dicentrarchus punctatus* and 160 *sparus aurata* " with different body weights (50- 600g) and lengths (10- 40cm) were randomly and seasonally collected from Lake Timsah in Ismailia Governorate during the period of the study (September 2020 – August 2021). They were transported alive to the laboratory of Fish Diseases and Management Department, Faculty of Veterinary Medicine, Suez Canal University in polyethylene bags containing 1/3 of their volume marine water, whereas the remaining volume was filled with air according to the method of **Randall (1983)**.

### **2. Clinical and postmortem examinations**

The body weight and total length of the investigated fishes were recorded before dissection using an electronic balance, followed by a clinical and P. M. evaluation of examined fishes. Fish specimens under inquiry were evaluated for clinical abnormalities and postmortem lesions, as stated by **Amlacker (1970)**.

### **3. Parasitological examination**

As soon as possible after sacrifice, fish specimens were examined macroscopically, following the method described by **Garcia et al. (2018)**, to detect any abnormalities in various parts of the fish body (skin, fins, gills, mouth cavity, gastrointestinal tract, musculature, kidney, liver, spleen, and heart) by naked eye and a hand lens. Internal parasites were examined microscopically.

### **4. Morphological analysis of isolated parasite**

**1) Nematodes:** The nematodes from the stomach and intestine were washed in saline before being relaxed and fixed in alcohol- glycerol 5. The worms were then cleaned in lactophenol, mounted in glycerin-gelatin, and studied microscopically after drying (**Meyer & Olsen, 1992**). Moreover, nematodes were identified according to the classification of **Ramachandran (1973)**.

**2) Digenetic trematodes:** The isolated trematodes were fixed using formalin 4%, stained with Semichon's acetocarmine, washed carefully in several changes of distilled water, dehydrated through a graded ethanol series (70–100%), cleared in clove oil, xylene, and mounted in Canada balsam then left to dry in horizontal position in hot air oven according to the method of **Negm-Eldin and Saleh (1995)**. Digenetic trematodes were identified according to **Yamaguti (1934)** and **Nahhas et al. (1998)**.

**3) Crustacea:** The observed adhered crustacean to the external body surface were washed with distilled water then fixed in 3% formalin, dehydrated through a graded ethanol series (70–100%), cleared in glycerin, mounted in glycerin-gelatin according to the guidelines of **Lucky (1977)** and then examined microscopically. Crustacean parasites were identified according to the classification of **Noga (1996)**.

### **5. Molecular detection and sequence analysis of *Anisakis simplex***

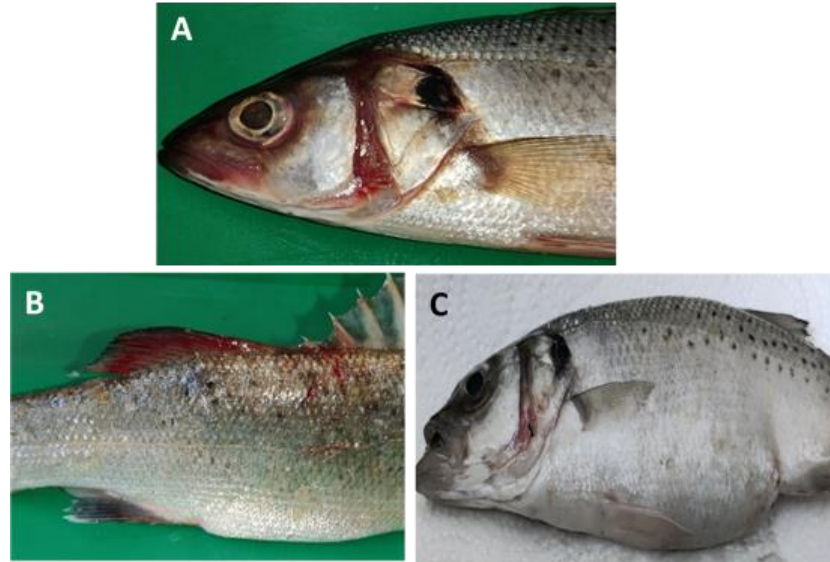
Following morphological identification, the DNA of *Anisakis simplex* was extracted using QIAamp DNA Mini Kit (Qiagen) according to manufacturer's instruction. PCR amplification for the NADH dehydrogenase subunit 1 (*ND1*) of mitochondrial (mt) gene was performed using the primer pairs (forward: 5'-TTCTTATGAGATTGCTTTT-3' and reverse: 5'-TATCATAACGAAAACGAGG-3'), as described by **Li *et al.* (2016)**. PCR was carried out using 50 $\mu$ L of a total reaction volume (2 X PCR Master Mix prepared according to Emerald Amp GT PCR mastermix (Takara) Code No. RR310A kit, Forward primer 20 picomole, Reverse primer 20 picomole, DNA extract 100 ng, Nuclease free water Up to 50 $\mu$ L). Standard cycle conditions for PCR were set as initial denaturation for 5min at 94°C, followed by 35 cycles of denaturation for 30s at 94°C, annealing for 40s at 50°C, and extension at 72°C for 45s, and a final extension at 72°C for 7min. To confirm the targeted PCR amplification, 5 $\mu$ L of the amplicons were separated by 1% agarose gel at constant 80V for 30min. The amplified product was visualized as a single compact band under UV light and documented by Samsung smart phone. Subsequently, a purified PCR product was sequenced in the forward and/ or reverse directions on an Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA). A ready reaction Bigdye Terminator V3.1 cycle sequencing kit was used. (Perkin-Elmer/Applied Biosystems, Foster City, CA), with Cat. No. 4336817. Sequences were then analyzed using BLAST®. Phylogenetic analysis was performed based on the ND1 mitochondrial gene. Sequences from several closely related fish nematode species were included. A phylogenetic tree was constructed in MEGA X using the neighbor-joining (NJ) method (**Tamura & Nei, 1993; Kumar *et al.*, 2018**).

## **RESULTS**

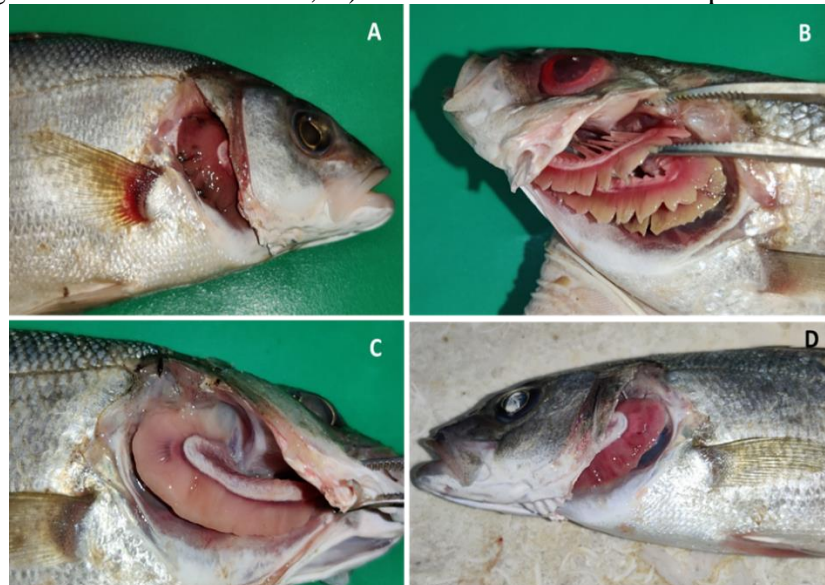
### **1. Clinical examination**

Most of the examined fishes (*D. labrax*, *D. punctatus* and *S. aurata*) showed no pathognomonic clinical abnormalities and were apparently normal. Some infested *D. labrax* showed hemorrhagic areas at mouth, operculum, around eyes and at fin bases (Fig. 1A, B), emaciation and somewhat abdominal distention (Fig. 1C), excessive mucus secretion with crustacean attachments and hemorrhages at pectoral fin bases, hemorrhagic eyes and sticking of gill filaments with greyish coloration, excessive mucus secretion with pale gills, and marbling appearance of the gills (Fig. 2A, B, C, D). Some

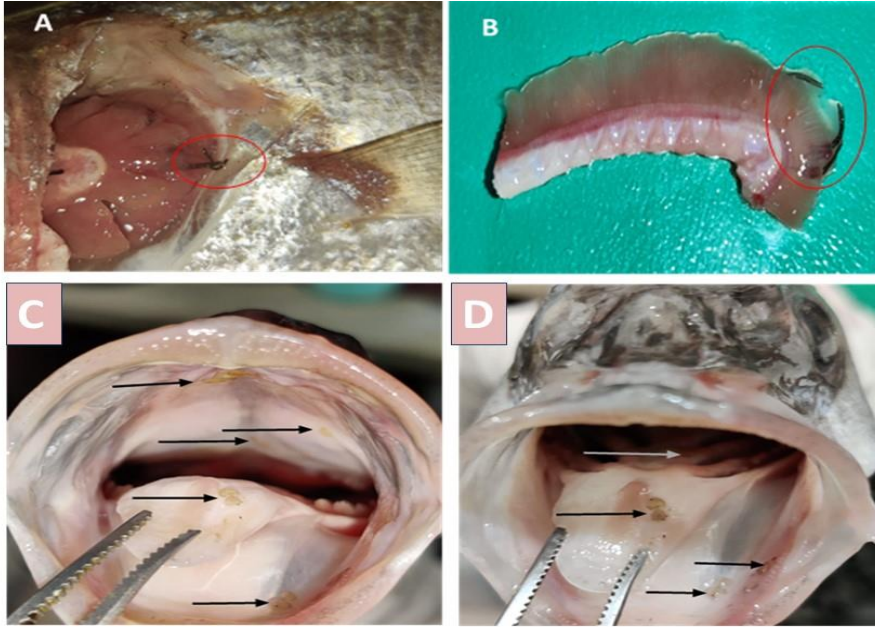
*D. labrax* gills revealed pale coloration with slimness and sticking of gill filaments with attachment of *Lernanthropus* sp. in form of black lines. In case of heavy infestation with *Caligus* sp., *D. labrax* showed attachment of the parasite to tongue, upper and lower palates, sides of the mouth and at gill rakers (Fig. 3A, B, C). Other cases showed up excessive mucus and mosaic appearance of gills with attachment of *Lernanthropus* sp., *Caligus* sp. and isopods (Fig. 3D).



**Fig. 1.** *D. punctatus* showing: **A)** Hemorrhages at head region; **B)** Hemorrhagic dorsal fin with slight abdominal distention; **C)** Abdominal distention and protrusion of the vent



**Fig. 2.** *D. labrax* showing **A)** excessive mucus secretion and hemorrhages at pectoral fin base, **B)** hemorrhagic eye and sticking of the gill filaments with greyish coloration, **C)** excessive mucus secretion with pale gills, **D)** marbling appearance of the gills



**Fig. 3.** *D. labrax* gills showing: **A & B**) Pale coloration with slimness and sticking of gill filaments and attachment of *Lernanthropus* sp. in the form of black lines (circles). **C**) Attachment of *Caligus* sp. to A. the tongue, upper and lower palates of the mouth (black arrows) **D**) Attachment of *Caligus* sp. to the sides of the mouth (black arrows) and at gill rakers (grey arrow)

## 2. Postmortem examination

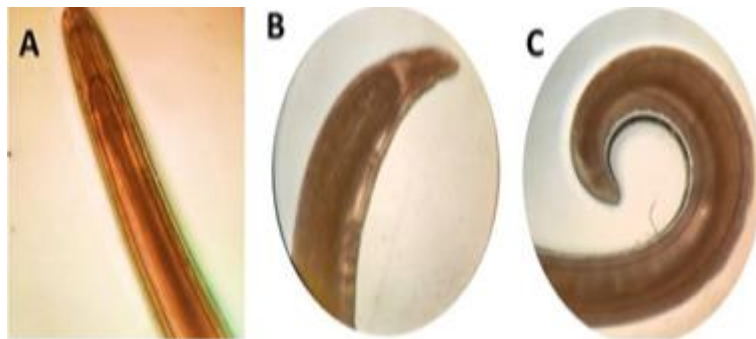
Naturally infested fish with external and/or internal parasites revealed no specific internal lesions. Some *D. labrax* infested with internal parasites exhibited pale gills and liver (Fig. 4A, C), while the liver showed mottling (Fig. 4B). Additionally, hemorrhaging was observed in the gills and kidneys of some infested fish (Fig. 4B). Some cases of *D. punctatus* revealed intestinal nematodes meanwhile, intestine exhibited congestion and inflammation of their walls with an excessive mucus secretion and attachment of trematode worms to the intestinal wall in few cases of *D. labrax*. In addition, there were nematode worms in the intestine of another *D. labrax* cases (Fig. 4D).

## 3. Morphology of isolated parasites

**Gastrointestinal nematodes *Hysterothylacium aduncum* (Rudolphi, 1802)** This worm was isolated from the intestine and stomach of *D. labrax* and *D. punctatus*. The front of the body is thinner. There are three lips and an interlabia; the lips are roughly as long as they are wide, with a small constriction at the anterior end; the interlabia is about half the length of the lips; and the characteristic "cactus tail" is present. The mature male has two equal spicules on the ventral side, while the adult female has a vulva opening toward the back of the first third of the body (Fig. 5A, B, C).

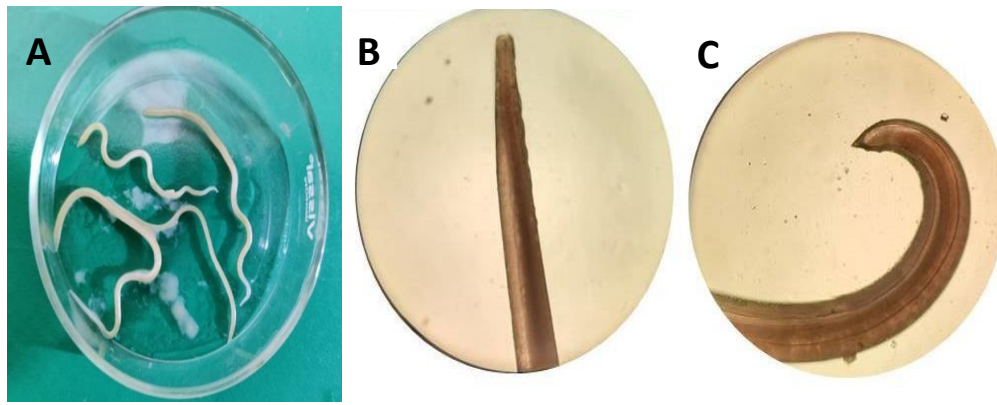


**Fig. 4.** **A.** *D. labrax* showing pale gills and liver; **B.** *D. punctatus* showing mottled liver and hemorrhagic gills and kidney (arrow); **C.** *D. punctatus* showing pale liver, **D.** *D. punctatus* showing intestinal nematode (arrow)



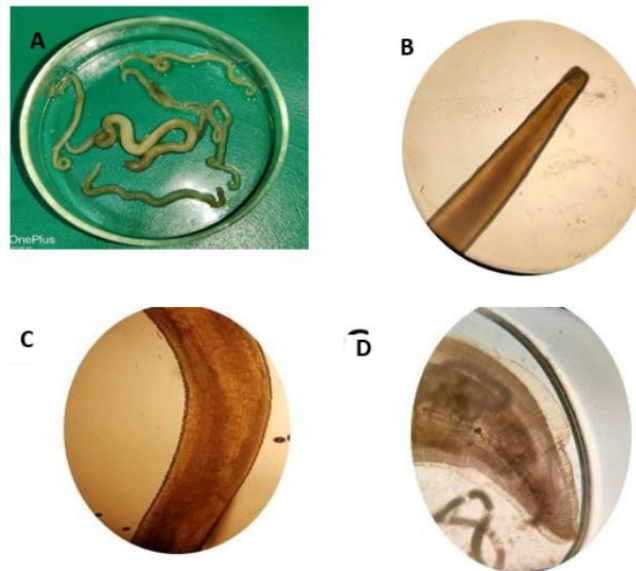
**Fig. 5.** A light photomicrograph of *Hysterothylacium aduncum*, **A.** anterior end; **B.** Caudal end of female; **C.** Caudal end of male

**Body cavity larval nematode *Anisakis simplex* third stage larva (Dujardin, 1845):** It was isolated from the body cavity of *D. labrax*, *D. punctatus* and *S. aurata*. When alive, the body is medium in size, white to cream colored. It was the thickest in the back, tapering progressively to the front. Three little lips surround the mouth, and a large boring tooth titles the body axis. An anus appears at the end of the intestine. The tail is short and rounded, with a tiny mucron at the end (Fig. 6A, B, C).



**Fig. 6.** A. A photograph of live *Anisakis simplex* larvae, a light photomicrograph of *A. simplex* B. anterior end, C. caudal end

**Gill larval nematode *Contraceacum* sp. third stage larva:** It was yellowish in color when alive, covered with a smooth transparent cuticle. The body was 15-35cm (average 23 cm) in length and 0.94-1.56cm (average 1.35 cm) in width. Three small lips with a prominent papilla and a well-defined boring tooth surrounded the mouth. Esophagus was narrow and long (Fig. 7).



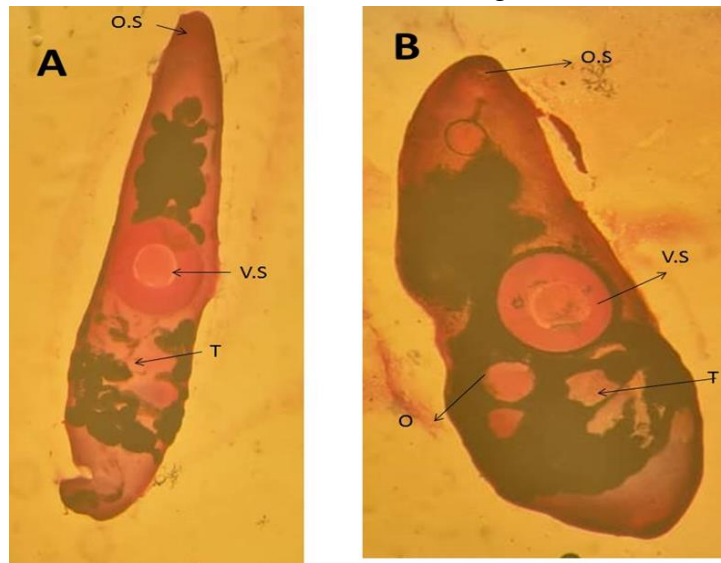
**Fig. 7.** A) A light photomicrograph of *Contraceacum* sp. larva B-C) anterior end, and D) posterior end

**Gastrointestinal digeneans *Erilepturus hamati* (Yamaguti, 1934):** They were isolated from stomach and intestine of *D. labrax*. The body is long, robust, and fusiform. The esophagus is a short and broad tube that connects the stomach. Two post-acetabular testes which are spherical, symmetrical, and approximately equal in size. The genital hole is located on the pharynx's posterior ventral border. The ovary is somewhat middle,



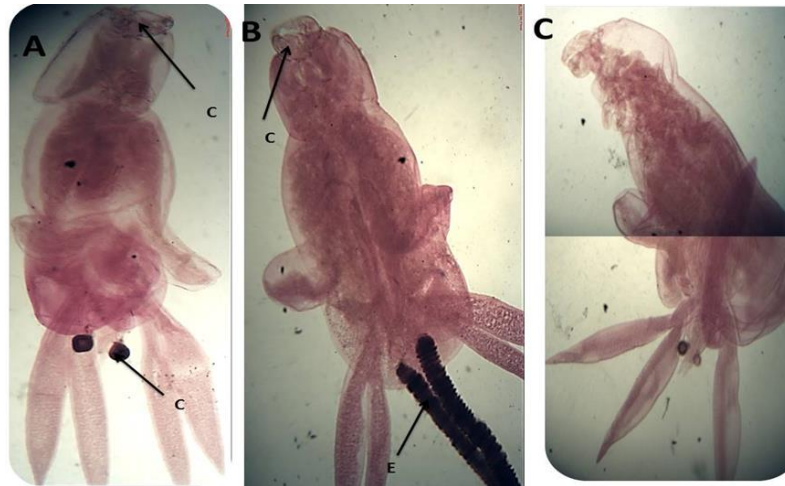
post-testicular, globular, and post-testicular. *Receptaculum seminis* highly developed and located behind the ovary. The uterus is coiled and muscular; the metraterm opens into the hermaphroditic duct. The eggs are small and numerous (Fig. 8A).

***Tangiopsis chinensis* (Shrjabin & Guschanskaja, 1955):** They were collected from the stomach and intestine of *D. labrax*. The body is rather massive, tapering towards the extremities, particularly the back. The maximum body width is 2.13mm, and the total body length is 5mm. The oral sucker is subterminal, round, and leads to a narrow throat. The ends of the intestines are joined posteriorly, making an arch at the level of the testes. The testes are big, oblique, and positioned postacetabularly. In front of the ventral sucker, the seminal vesicle is turned back on itself (Fig. 8B).



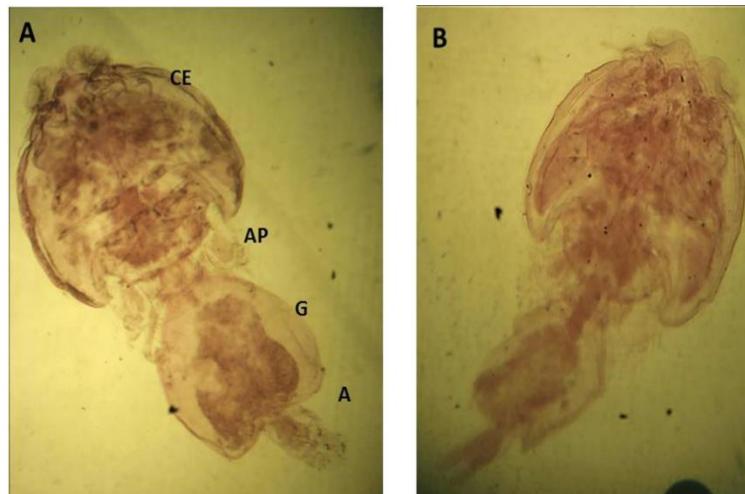
**Fig. 8.** A light photomicrograph of stained digenea **A)** *Erilepturus hamate*, **B)** *Tangiopsis chinensis*, OS: Oral sucker, VS: Ventral sucker, O: Ovary, T: testes

**Copepod species *Lernanthropus koryeri* (Beneden, 1851):** It was isolated from the gills of both of *Dicentrarchus labrax* and *Dicentrarchus punctatus*. The cephalon and the first thoracic segment of both female and male fused to form a cephalothorax that is somewhat wider than long in both sexes. The cephalothorax is narrower anteriorly with a dorsal shield curved ventrally on each side in female end and flat in male. Two dorsolateral conspicuous sutures split the cephalothorax into a big posterior thoracic plate and a small anterior cephalic plate. The thoracic appendages are larger than the first and second thoracic legs, which culminate with hand-like spines. Females were distinguished by egg-strings visible on the gills (Fig. 9).



**Fig. 9.** A light photomicrograph of *Lernanathropus kroyeri* A) Whole male copepod, B) Whole female copepod, and C) lateral view of male copepod. c: claws, E: egg sac, s: spermatophore (Beneden, 1851)

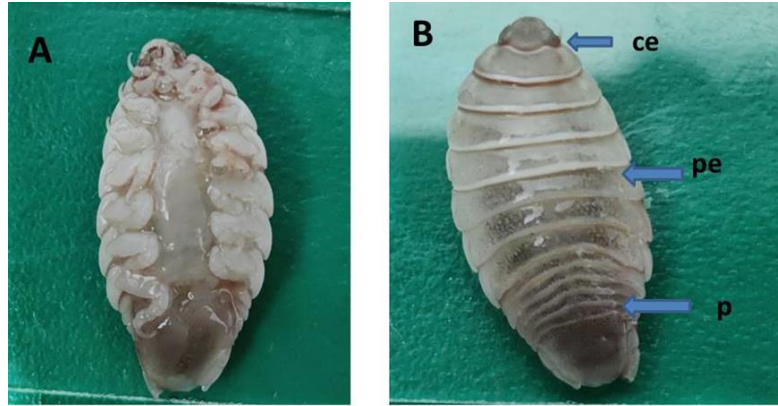
***Caligus minimus* (Otto, 1821):** It was isolated from the inner surface of the operculum of *Dicentrarchus labrax* and *Dicentrarchus punctatus*. The posterior section of the cephalothorax is joined with an apron, which includes third leg and the tagma. The genital complex is made up of the genital segment and the fourth leg-bearing segment of the thorax. The intestine, immature eggs and oviduct channel are all present in the genital section. The abdomen and caudal rami were the last parts of the *C. minimus*. Egg column, mature, and immature eggs were also discovered. The eggs have a cylindrical flattened form (Fig. 10A, B).



**Fig. 10.** (A&B) A light photomicrograph of *Caligus minimus*; CE: cephalothorax; AP: Apron; G: Genital segment; A: Abdomen

**Isopod species *Livoneca redmanii* (Leach, 1818):** It was isolated from *D. punctatus*. The body is ovoid and typically twisted to one side, with dark chromatophores and a

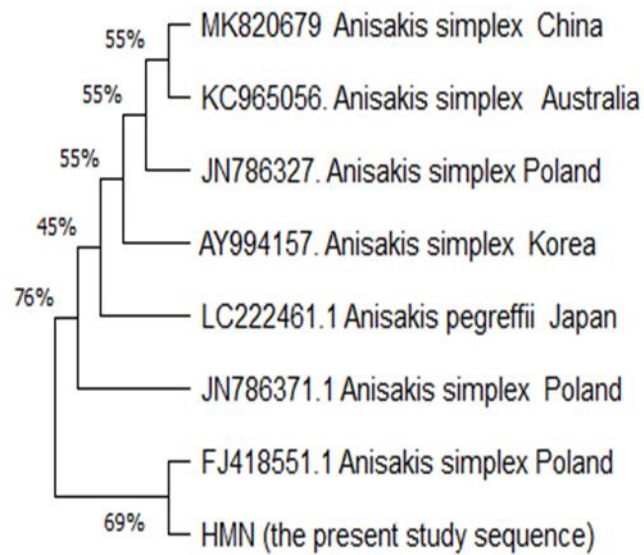
light brown color. Cephalon does not project between the antennae's bases. It has a trilobed posterior border. Laterally, one pair of eyes can be found. Pereopods have two pairs of antennae, the last two of which are narrower. Pereopods are strong and have massive dactyli. Pleon is narrower than pereon and is not submerged in it. It is made up of six parts that gradually narrow in width as they approach the back (Fig. 11A, B)



**Fig. 11.** A photograph of *Livoneca redmanii*, A. Ventral view; B. Dorsal view, ce: Cephalon, pe.: Pereon, p: Pleon

#### 4. Molecular identification of *Anisakis simplex*

The samples were analyzed and identified as *Anisakis simplex* larvae based on ND1 of mitochondrial mt gene. The DNA sequences were blasted in the GenBank and identified as *Anisakis simplex* larva. The dendrogram was constructed using a neighbor-joining (NJ) method. In NJ method, each group was divided into two lineages, and the analysis revealed the closest relationship between the sequences obtained in the current study and the *Anisakis simplex* which was isolated from Poland as they clustered together, sharing the same ancestor. The rest of the *Anisakis* species are clustered together and are far from our sequences (Fig. 12).



**Fig. 12.** The phylogenetic analysis was constructed using neighbor-joining (NJ) method to construct the phylogenetic tree of some of Anisakis species sequences from the Genbank, and our sequence samples are included based on ND1 sequences

### 5. Prevalence of parasitic infestations

Total prevalence of parasitic infestations among the examined fishes was 57.91% (Table 1). The highest percentage was recorded in *Dicentrarchus punctatus* (74.38%), followed by *Dicentrarchus labrax* (71.25%) and *Sparus aurata* (28.13%).

**Table 1.** Prevalence of different parasitic infestations among the examined fishes

Infestation Fish species	Nematodiasis	Digeneasis	Crustacean infestation
<i>D. labrax</i> (n=160)	22 (13.75%)	4 (2.5%)	88 (55%)
<i>D. punctatus</i> (n=160)	18 (11.25%)	0 (0%)	101 (63.12%)
<i>Sparus aurata</i> (n=160)	45 (28.12%)	0(0%)	0(0%)
Total (n=480)	85 (17.70%)	4 (0.83%)	189 (39.37%)

Crustacean species prevalence appeared in the examined fish species, as showed in the data presented in Table (2).

**Table 2.** Prevalence of different crustacean infestations among *D. labrax* and *D. punctatus*

Infestation Fish Species	<i>Lernanthropus koryeri</i>	<i>Caligus minimus</i>	<i>Livoneca redmanii</i>
<i>D. labrax</i> (n=160)	50 (31.25%)	38 (23.75%)	0(0%)
<i>D. punctatus</i> (n=160)	47 (29.37%)	51 (31.87%)	3 (1.27%)
Total (n=320)	97 (30.31%)	29 (27.81%)	3 (0.94%)

Statistical analysis of the total parasitic infestation rate among different fish species across seasons revealed significant differences in infestation rates ( $P<0.001$ ). The highest rate was observed in winter (81.6%), followed by spring (70.8%), autumn (55%), and summer (24%), as shown in Table (3).

**Table 3.** Seasonal prevalence of parasitic infestation among examined fishes

Season Fish	<i>D. labrax</i> n=40/season	<i>D. punctatus</i> n=40	<i>Sparus aurata</i> n=40	Total n=120
Autumn	34 (85%)	28 (70%)	4 (10%)	66 (55%)
Winter	33 (82.5%)	35 (87.5%)	30 (75%)	98 (81.6%)
Spring	36 (90%)	38 (95%)	11 (28%)	85 (70.8%)
Summer	11 (27.5%)	18 (45%)	0 (0%)	29 (24%)

The nematode infestation recorded the highest rate of 45.8% in winter, and the lowest was in summer with no recorded infestation. The total prevalence of digeneans among the examined fish showed no significant difference statistically ( $P>0.05$ ). Spring had the highest infestation rate at 3.30% (4/120), while no infestations were observed in other seasons. Regarding crustacean infestations, the highest prevalence was observed in autumn (50%), followed by spring (47.50%), winter (35.80%), and summer (24.20%), as shown in Table (4).

**Table 4.** Seasonal prevalence of different crustacean infestations in *D. labrax* and *D. punctatus*

Infestation \ Season	<i>D.Labrax crustacean infestations</i>			<i>D.punctatus crustacean infestations</i>		
	<i>nanthropus kroyeri</i>	<i>Caligus minimus</i>	<i>Livoneca redmanii</i>	<i>nanthropus kroyeri</i>	<i>Caligus minimus</i>	<i>Livoneca redmanii</i>
Autumn	21 (52.5%)	11 (27.5%)	0 (0%)	16 (40%)	12 (30%)	0 (0%)
Winter	13 (32.5%)	8 (20%)	0 (0%)	11 (27.5%)	11 (27.5%)	0 (0%)
Spring	7 (17.5%)	17 (42.5%)	0 (0%)	10 (25%)	21 (52.5%)	2 (5%)
Summer	9 (22.5%)	2 (5%)	0 (0%)	10 (25%)	7 (17.5%)	1 (2.5%)
Total	50	38	0	47	51	3

## DISCUSSION

Parasitic infestations are responsible for the majority of infectious diseases affecting fish. The primary goal of this research is to identify the prevalent parasitic diseases affecting certain marine fish species (*Dicentrarchus labrax*, *Dicentrarchus punctatus*, and *Sparus aurata*). This study found hemorrhagic patches on the body surface of *D. labrax*, particularly around the mouth, on the gill cover, and at the base of the fins. These findings are consistent with those of **Eissa *et al.* (2012)**. These lesions may be attributed to significant irritation caused by movement, feeding habits, crustacean claw adhesion, and mucus acting as a defense mechanism against infestation and irritation (**Abdel-Mawla & El-Ekiaby, 2012**).

Abdominal distension and emaciation were the most common clinical signs observed in fish infected with internal parasites (digeneans and nematodes). These symptoms result from the impact of parasites on the gastrointestinal tract, leading to tissue destruction, modification, and mechanical blockage that impairs nutrient absorption (**El-Iamie, 2007**).

In *D. labrax* infested with digeneans, hemorrhagic regions at the operculum, abrasion, ulceration of fins, and abdominal distention were reported. In the presence of isopods, opercular bulging can occur, potentially leading to the sloughing of gill filaments in one or two gill arches, as well as skin lesions visible around the operculum after isopods were removed. This is supported by the studies of **Eissa (2002)** and **Abd El Aal and Ashram (2011)**.

Infested *Dicentrarchus labrax* and *Dicentrarchus punctatus* have pinpoint or ulcerative ulcers inside their mouths, especially in cases of extensive sea lice infestation. Multifocal hemorrhagic patches were found on the tongue and palate of the infested fishes' upper jaw. In other cases, a significant infestation of sea lice resulted in severe hemorrhages on the outside of the mouth and even into the buccal canal. The acute irritation caused by the mobility and feeding activities of sea lice could account for these results. This finding is in line with those of **Price *et al.* (2011)** and **Noor El-Deen (2013)**. Sea lice were only identified in the buccal cavities of the studied fishes; none was found on the fishes' exterior body surfaces.

When *D. punctatus* was infected with isopoda; there were exceedingly pale gills, gill damage, and excessive mucus secretion when big sections of filaments were damaged. It is so harmful that isopods can change their feeding habits and feed on the gill filaments, killing their host. These lesions may be attributed to the intense irritation induced by movement, feeding habits, crustacean claw tight adhesion, and mucus acting as a defense mechanism against infestation and irritation. Furthermore, the pressure exerted by big parasites frequently causes mechanical damage and atrophy of lamellar structures, affecting opercular respiratory motions, as postulated in previous studies (**Panakkool-Thamban *et al.*, 2015; Rania & Rehab, 2015; Ali and Aboyadak, 2018**).

In heavy infestations of *D. labrax* and *D. punctatus*, *Lernanthropus kroyeri* was apparent by the naked eyes, generating black lines between the gill filaments, increased mucus secretion, hemorrhagic lines, and anemic gill filaments. This finding is supported by the studies of **Woo and Leatherland (2006)** and **Yardimci and Pekmezci (2012)**. Furthermore, there was a note about the female parasite's preferred site, which was the deep area between the hemibranchs of the second gill arch of *D. labrax*, and was usually absent in the first. This result is similar to that of **Toksen (2007)**, who found that the female parasite prefers the deep area between the hemibranchs of the second gill arch. Male parasites were found on the posterior hemibranchs next to females, although they were rarely seen in filaments of the first and third gill arch.

In the case of *D. labrax* and *D. punctatus*, heavy infestation of *Caligus* sp. was observed on the inner surface of the operculum (upright position), in the branchial cavity, on the gill rakers and in the buccal cavity. This result agrees with the findings of **Ragias *et al.* (2004)** and **Helna *et al.* (2018)**. However, it contradicts that of **Hvidsten *et al.* (2007)**. It's possible, though, that badly afflicted fish perish and aren't detected. This disparity could be attributable to Caligidae species, the type of fish analyzed, and the region where the fish were gathered.

There was emaciation and some abdominal distension in the studied fishes due to an internal parasite infestation. This is related to the parasite influence on the gastrointestinal tract, which causes tissue modification and destruction, a reduction in gut absorption, and mechanical blockage (**Eissa *et al.*, 2010**).

Regarding *Lernanthropus kroyeri* (Van Beneden, 1851), isolated from the gills of *Dicentrarchus labrax*, the results are supported by the studies of Manera and Dezfuli (2003), Toksen (2007), Samak and Ashraf (2008) and Yardimci and Pekmezci (2012). The isolation of *Lernanthropus kroyeri* from the gills of *Dicentrarchus punctatus* is consistent with that of El-Boghdady *et al.* (2015), who also found this parasite in both *D. labrax* and *D. punctatus*. This is in line with the study of Sharp *et al.* (2003), who noted that many *Lernanthropus* species parasitize multiple fish species within a genus or across genera within a family.

For *Caligus minimus* (Otto, 1821) isolated from *D. labrax*, its presence in *D. punctatus* agrees with the findings of Paperna (1980) and is confirmed by Noor El-Deen *et al.* (2013). Additionally, *Hysterothylacium* spp. larvae of nematodes isolated from *Sparus aurata* are supported by Kalay *et al.* (2009). *Tangiopsis chinensis* collected from the stomach and intestine of *D. labrax* aligns with Qorany (2020), who also isolated this species from the stomach of *D. labrax*. *Erilepturus hamati* isolated from the stomach and intestine of *D. labrax* is consistent with the outcome of Qorany (2020).

*Livoneca redmanii* was isolated from the gills of *D. punctatus*, matching the findings of Qorany (2020). However, it contradicts that of Helal and Yousef (2018), who reported it from the skin and gills of *Mugil cephalus*. *Anisakis simplex* third-stage larvae were isolated from the body cavity of *D. labrax*, *D. punctatus*, and *S. aurata*. *Contracaecum* spp. third-stage larvae isolated from *S. aurata* gills contradict Abo-Esa (2007), who found these larvae in the stomach and intestine of *Lutjanus* species, and Abdel-Mawla and Yousef (2018), who isolated them from the stomach and intestine of *Trachurus indicus*.

Hemorrhages, excessive mucus secretion, hemorrhagic striations, and attachment of trematode worms to the intestinal wall in *D. labrax* are consistent with the findings reported by Eissa (2002). Postmortem examination revealed hemorrhagic livers, slightly marbled gills with excessive mucus secretion, and slight abdominal bulging, similar to the observations of Eissa (2002) and El-lamie (2007). Hemorrhagic regions on the gill cover, belly, and fin bases match the findings of Ragias *et al.* (2004) and Khalil *et al.* (2014).

According to Tavares and Luque (2001) and Nagasawa (2004), emaciation in *D. labrax* may be linked to crustacean infestations, which reduce fish appetite. Postmortem examination of the gills revealed congestion, paleness or marbling, profuse mucus secretion, and adherence of gill tips with greyish staining. Both *Dicentrarchus labrax* and *Dicentrarchus punctatus* showed greyish color, sticky gill tips, and profuse mucus secretion upon postmortem examination. The gills had a marbled (mosaic) appearance, with areas of congestion and paleness. This could be attributed to the parasites' mobility and feeding activities, causing a significant irritation. Excessive slime production might be a defense mechanism used by the host to expel the parasites. Gill marbling results from blocked blood flow in the branchial filaments due to feeding



pressure and hypertrophy from the parasites. In some cases, parasites can be detected by the naked eye as black lines among the gill filaments, such as egg sacs of copepods. This observation is supported by the studies of **El-Lamie (2007)**, **Abdel-Mawla and El-Ekiaby (2012)**, and **Noor El-Deen *et al.* (2013)**.

In the present study, the total prevalence of parasitic infestation is 57.91%. This result is higher than that obtained by **Yussef and Derwa (2005)** which was 41, 51, 15.7 and 22%, respectively, while it is lower than that obtained by **Mousa *et al.* (2015)** and **Zaid *et al.* (2018)** among marine fishes which was 96, 79.5 and 87%, respectively. This may be due to differences of the examined fishes and the locality from which fishes were obtained. In addition, the prevalence of the parasitic infestation in *D. labrax*, *D. punctatus* and *S. aurata* are 71.25, 74.38 and 28.13%, respectively. *S. aurata* has the lowest prevalence; this may be due to the difference in natural habitats of the three different fish species. *D. labrax* and *D. punctatus* are demersal fishes living in the seabed and migrate to brackish water, therefore their presence give the parasite a good chance to infect them, whereas *S. aurata* lives in seagrass beds and sandy bottoms at depths of about 30m and up to 150m and does not migrate to the brackish water, which resulted in reducing the possibility of parasitic infestation (**Qorany, 2020**). Regarding the total prevalence of nematodiasis among examined fishes (17.70%), this result is higher than that obtained by **Genc (2002)**, **Kalay *et al.* (2009)** and **Qorany (2020)** with values of 1.74, 3.33, 6.25, 1.25, and 0.7%, respectively. While this result is lower than those obtained by **El-Ekiaby (2009)** and **Abdel-Mawla and Yousef (2018)**, which were 65.81, 28. and 39%, respectively. This variation may be due to the different localities, which usually enhance or limit the parasitic spread, the different sources of the examined fish, the age, the sex of the examined fish. In *D. labrax*, it was 13.75%. This disagrees with **El-Lamie (2007)** as it was 3.33% in *D. labrax*. While in *D. punctatus*, it was 11.25 and 28.12% in *Sparus aurata*. This result is lower than that obtained by **Kalay *et al.* (2009)** and **Qorany (2020)** as it was 6.25, 2.15%, respectively, from seabream. This difference may be due to the locality of collection. Concerning the total prevalence of digeniasis, it is 0.83%. This result is lower than that obtained by **Mohamed (2017)** and **Qorany (2020)** from marine fish farms (43.5%) and the Suez Canal area (7.34%), respectively. This may be due to the culture condition and locality differences. *D. labrax* shows 2.5% with no infestation in *D. punctatus* and *Sparus aurata*. This result is lower than that obtained by **Emre *et al.* (2014)**, **Abdel-Mawla and Yousef (2018)** and **Qorany (2020)**, who recorded 10.3, 32.14, 7.34% respectively. This may be attributed to the type of the examined fish and the locality from which fish were obtained. In this study, the total prevalence of crustacean infestation is 39.37%. This result is lower than that obtained by **Elzoghby (2020)** as it was 50.41%. Moreover, it is higher than that obtained by **Adel (2017)** which was 21%. This difference may be ascribed to the difference in the examined hosts and localities. Percentages of 55 and 63.12% were recorded for *D. labrax* and *D. punctatus*, respectively. This result is higher

than that obtained by **Hassanin (2016)**, who reported 48.57% in *D.labrax* and lower than that obtained by **Elzoghby (2020)** with 62.91% in *D. labrax*. This may be traced back to the locality difference. Regarding different crustacean infestations in *D. labrax* and *D. punctatus* together, firstly the total prevalence of *Lernanthropus kroyeri* is 30.31%. This result is higher than those recorded in the studies of **Noor El-Deen *et al.* (2013)**, **Zaid *et al.* (2018)** and **Elzoghby (2020)**, with values of 20, 18, and 4.16%, respectively. Conversely, it is lower than that obtained by **Toksen (2007)**, **Aneesh *et al.* (2014)** and **Qorany (2020)** who recorded values of 100, 81.4 and 43.36%, respectively. These differences may be related to the locality from which the fish were obtained and the differences in type, number and size of the fish. In the current study, specifically in *D. labrax*, it was 31.25%. This result is higher than that obtained by **Elzoghby (2020)** as it was 12.5% in *D. labrax*. On the other hand, in *D. punctatus*, it was recorded as 29.37%, which is higher than that obtained by **Bahri *et al.* (2002)** with 25% and lower than that obtained by **El-Boghdady *et al.* (2015)** and **Qorany (2020)** who reported values of 41% and 66.34%, respectively. In this study, *D. labrax* has an increased prevalence compared to *D. punctatus*. This is explained by **Bahri *et al.* (2002)**, who recorded that *L. kroyeri* was more frequent on the common bass than on the spotted seabass. Secondly, the total prevalence of *Caligus minimus* was recorded at 27.8%. This result is higher than that obtained by **Qorany (2020)** with a value of 10.14% and lower than that of **Elzoghby (2020)** who reported a value of 33.47%. This difference may be attributed to the difference of both locality and the collected fish species. In *D. labrax*, it was recorded at 23.75%. This value nearly agrees with that of **Elzoghby (2020)**, who noted a value of 24.16 % in *D. labrax*. Furthermore, it is lower than that of **Akif and Kayis (2015)**, who recorded 94% in the same fish. Whereas, in *D. punctatus*, it was recorded with a value of 31.87%. This result is higher than that obtained by **Elzoghby (2020)**, as it was 22.91% among *Mugil cephalus*. This may be due to the difference in fish species and locality. Thirdly, the total prevalence of *Livoneca redmanii* was 0.94%. This is lower than that obtained by **Elzoghby (2020)** as it was 5.13%. This may be attributed to the fishermen interference, as they told me that they removed the isopods from fish once they found them accidentally to avoid marketability affection, hence this low result may not be attributed to natural affection. It was 1.87% in *D. punctatus* while there was no infestation in *D.labrax*. This result is lower than that of **Elzoghby (2020)** which was 3.33 and 12.08% in *D.labrax* and *M.cephalus*, respectively. In this study, the seasonal prevalence of parasitic infestations among all 3 examined fishes was the highest in winter (81.6%), followed by spring (70.8%), autumn (55%) and summer (24%) since they showed significant difference of infestation within different seasons. This variation between seasons may be attributed to the change in the climatic conditions. This sequence agrees with that of **El-Lamie (2007)**, who recorded the highest infestation in winter (81.3%), followed by spring (78.6%) then autumn (66.6%), while the lowest was in summer (53.3%); on the other hand, this outcome disagrees with

both **El-Boghdady *et al.* (2015)** and **Hassan (2017)**, who recorded the highest prevalence in summer (66%) and autumn (80%) and the lowest in autumn (48%) and winter (20%), respectively. Meanwhile, this result is nearly similar to the findings of **Qorany (2020)**, who recorded the lowest prevalence in summer (43.14%) but disagrees with the result of the highest infestation season as it was the autumn (60.71%). Regarding the total nematode infestation in different seasons, it was the highest in winter 45.8% and the lowest in summer 0% with a significant difference between seasons. The total prevalence of crustacean infestation in different seasons had significant difference ( $P<0.001$ ) as the highest value was recorded in autumn (50%) and the lowest was in the summer season (24.2%). This sequence nearly agrees with **Noor El-Deen *et al.* (2013)**, who recorded the highest infestation rate during autumn and spring (86%) and decreased during winter and summer. In addition, it is similar to that of **Elzoghby (2020)** which had the highest in autumn (72.22%) but not similar in terms of the lowest season, being winter (28.33%)

In this study the samples were analyzed and identified as *Anisakis simplex* larvae based on ND1 of mitochondrial mt gene with product at an approximately 370bp. This result coincides with the finding of **Li *et al.* (2016)**, who used molecular detection and phylogenetic analysis of ND1 gene of *Toxocara vitulorum*. Additionally, this finding agrees with that of **Abdel-Mawla and Yousef (2018)**, who isolated and identified *Anisakis* larvae isolated from *Saurida undosquamis* through *Anisakis* ITS gene. Therefore, molecular identification is an important method in parasite identification.

## CONCLUSION

The present study was directed toward the investigation of the most prominent parasitic diseases affecting some marine fishes at Lake Timsah. Further studies should be done for controlling these parasites.

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