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Pathological studies on some marine fish parasites in Egypt

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ABSTRACT

Marine fish is an important source of high-quality, yet relatively cheap animal protein in the Egyptian's diet. Although most parasitic diseases might not cause direct losses of fish, it has deleterious effects on their weight gain and marketability and weaken the fish's immune system paving the way for more serious secondary infections. In this study, we recorded the most common parasitic infestations in marine fish in Egypt in the period between winter 2019 to summer 2021 and described their associated pathologic effects. Four hundred fish from seven different fish species (*Mugil cephalus*, *Pomadasys incisus*, *Gilthead sea bream*, *Pagrus Pagrus*, *Morone labrax* (sea bass), *thunnus thynnus* and *Caranx crysos*) were randomly collected from the Mediterranean and the Red Sea. Out of the 400 examined fish, 197 (49.25%) infestations were recorded. The isolated parasites were a crustacean, two cestodes, and a nematode and were morphologically identified as follows: *Ceratothoa oestroides*, *Callitetrarhynchus gracilis*, *Tetraphyllidean* larvae and *Hysterothylacium deardorffoverstreetorum*). Among the infested fish, the highest prevalence of infestation was recorded by *Ceratothoa oestroides* (43.65%) followed by *Hysterothylacium deardorffoverstreetorum* (31.98%) and *Callitetrarhynchus gracilis* (24.37%), and the lowest prevalence rate was recorded by the *Tetraphyllidean* larvae (15.74%). The pathologic macroscopic and microscopic lesions associated with each type of infestation are described underscoring the importance of continuous search for and application of possible safe control measures for marine fish parasites to spare the fish industry the detrimental effects of these infestations.

Introduction

Fish and fishery products provide more than one third of the total protein consumption in Egypt and fish consumption per capita is increasing (Tran *et al*., 2016). Such reliability on fish for food security requires that we take control over all the hazards that can harm this industry. Environmental pollution and the resulting reduced water quality is having its toll on the marine resources. With the stress this brings on fish, the immune system is exhausted and fish is more prone to infections. Infectious diseases of fish are generally classified as bacterial, viral, fungal and parasitic diseases. Some bacterial and viral infections can induce high mortalities, on the other hand, parasites are excellent indicators of the fishery environmental quality (Jerônimo *et al*., 2022) and they can harm their hosting fish by causing mechanical damage, competing for their nutrients, intoxicating them with their excreted metabolic products or lowering their immunity and facilitating secondary infections (Klimpel *et al*., 2019).

Marine teleosts can become final or intermediate hosts for a wide range of parasitic infestations Helal and Yousef (2018) and Fahmy (2020) reported a rise in the Cymothoid isopods infestations in lake Qarun in Egypt in the last decade. This crustacean is an ectoparasite that attach to the skin or gills and sometimes the buccal cavity of the infested fish causing hindrance to the respiration, severe irritation to the skin and can lead to mortalities of young fish in case of heavy infestations (Horton and Okamura, 2001).

Eissa *et al*. (2020) reported a high prevalence of parasitic infestation (66%) among marine fish collected from Suez Canal in Egypt with the highest prevalence recorded by cestodal larvae and molecular identification confirming the larvae belongs to the Genus *Callitetrarhynchus* which belongs to the order *Trypanorhyncha*. The *Trypanorhynchid* larvae infest a large variety of fish and are morphologically identified by their characteristic scolex with two or four bothria and four hooked tentacles (Jones *et al*., 2004). The larval migration and encystation of the *Trypanorhyncha plerocercoids* is associated with development of migratory tracks in the viscera particularly the liver and degeneration and atrophy of internal organs (Ibrahim, 2000). Nematodal infections of fish are probably the most problematic due to zoonotic significance of some members of the family Anisakidae. Khalifa *et al*. (2019) described six types of Hysterothylacium larvae isolated from the Red Sea fish.

The main purpose of this study was to identify the most prevalent parasites infesting marine fish randomly collected from the Egyptian coasts in the period between winter 2019 to summer 2021 and provide full description of the pathologic effects of infestation on the hosting fish.

Materials and methods

Ethical approval

The study was designed with the permission of the Regulations of an Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine ((ZU-IACUC/2/F/2023)), Zagazig University, Egypt.

Study area

Fish were collected from the Red Sea in the east and the Mediterranean Sea and salt lakes in the north (Alexandria, Ismailia, Almanzala, Port Said, Suez and Hurghada).

Fish samples

Four hundred random samples from seven different fish species were collected from the previously mentioned localities in the period be-

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tween winter 2019 to summer 2021 as follows: *Mugil cephalus* (n=62), *Pomadasys incisus* (n=48), *Gilthead sea bream* (n=40), *Pagrus Pagrus* (n=62), *Morone labrax* (sea bass) (n=41), *thunnus thynnus* (n=75) and *Caranx crysos* (n=72)). Body weight of the collected fish samples ranged from 1.48 to 8 kg..

Parasitological examination

External examination for crustaceans and other external parasites was done grossly. Isopod samples were recovered from both branchial cavity and skin of infested fish then fixed in AFA (alcohol formalin acetic acid) according to Woodland (2006). Fixed isopods were measured and classified based on their morphological features according to Bruce (2004).

Internal examination was carefully applied on flesh, stomach wall, intestine wall, mesenteries, and body cavity for cestodes, nematodes and/or trematodes.

The isolated cestodes (*Trypanorenchya* and *Tetraphyllidean* larvae) were washed in saline solution and fixed in 10% buffered formalin. Specimens were stained in acetic carmine, dehydrated and then mounted by Canada balsam and cover-slipped. *Trypanorenchya* identifications and classification were done according to Palm (1997) *Tetraphyllidean* larvae identifications and classification were done according to Santoro *et al*. (2013)

The nematode larvae (anisakid larvae) that were collected were fixed, clarified and preserved as described by Knoff *et al*. (2012). The morphological analysis to identify *Hysterothylacium deardorffoverstreetorum* larvae was performed according to Kuraiem *et al*. (2017).

Prevalence, identification and classification of isolated parasites from examined fish were recorded carefully.

Pathological examination

The collected fish tissues and gills were taken from the parasite attachment area of infested fish in fresh condition, trimmed and fixed in 10% buffered neutral formalin. Tissues were then washed in tap water and dehydrated using graded alcohol concentrations and subsequently cleared in xylol and embedded in paraffin wax. Serial sections 3–5-micron thickness were cut using a rotating microtome and stained with Mayer's haematoxylin and Eosin for histo-pathological examination (Suvarna *et al*.., 2013)

Results

One hundred and ninety-seven marine fish out of 400 (49.25%) randomly selected fish were infested with parasites. The highest prevalence rate of infestation was during the summer season of 2021 with *Isopoda* sp. (*Cymothoidae oestroides*) in five species of marine fish 86/197 (43.65%) representing the highest prevalence of infestation followed by Hysterothylacium sp. (H. deardorffoverstreetorum) with prevalence rate 63/197 (31.98%) isolated solely from *Caranx crysos*. *Trypanorenchya* larvae (*Callitetrarhynchus gracilis*) prevalence rate was 48/197(24.37%) and were isolated from *thunnus thynnus* and *Pagrus Pagrus* fish in summers of 2019 and 2020. The lowest prevalence rate was recorded by *Tetraphyllidean* larvae (*T. plerocercoid*) with prevalence rate 31/197(15.74%) mostly during the summers and autumns of both 2019 and 2020. Mixed infestation was recorded in 31 fish. Seasonal prevalence, identification and classification of isolated parasites from examined fishes were fully demonstrated in (Table 1) while photomicrographs of isolated parasites were declared in (Fig. 1).

Clinical examination of fish

The examined marine fish revealed some infestations with *Crustacean Isopoda* (*Cymothoidae oestroides*) either attached to skin or protruding from gill operculum while fish showed restlessness, scratching their bodies against the wall of the fishing aquaria. In addition, they also showed slow swimming mostly at the water surface with excessive mucus secretion and acceleration of operculum movement. The fish infested with Trypanorenchea larvae (*Callitetrarhynchus gracilis*) showed slow movement and in severe cases twisting was noticed with enlarged abdomen in heavy infestations. The fish infested with both *Tetraphyllidean* larvae and *Hysterothylacium deardorffoverstreetorum* had no clear clinical signs.

Pathological findings

Ceratothoa oestroides

These crustacean parasites were found either attached to the skin or protruding from the gills below the operculum causing its bulging unilaterally or bilaterally (Fig. 2 A-B). Grossly, the infested gills were mainly congested and eroded with increased mucus secretion, but sometimes

Table 1. Seasonal prevalence, identification and classification of isolated parasites from examined fish.

Fish species and numbers	*Mugil cephalus* (n = 62)	*Pomadasys incisus* (n = 48)	*Gilthead (sea bream)* (n = 40)	*Pagrus Pagrus* (n = 62)	*Morone labrax (sea bass)* (n = 41)	*Thunnus thynnus* (n = 75)	*Caranx crysos* (n = 72)	Total fish (n = 400)
Parasite species								
Isopda sp. (Cymothoidae oestroides)	22 fish Summer 2021	16 fish Summer 2020, 2021	5 fish Summer 2019, 2021	26 fish Summer 2020, 2021	17 fish Summer 2019, 2020, 2021	(-)	(-)	86
Trypanorenchea larvae. (Callitetrarhynchus gracilis)	(-)	(-)	(-)	12 fish Summer 2019, 2021	(-)	36 fish Summer 2019, 2020, 2021	(-)	48
Tetraphyllidean larvae.	2 fish Summer 2019, 2021	(-)	4 fish Summer 2019, 2021	(-)	(-)	11 fish Summer 2019, Autumn 2020	14 fish Autumn 2019, Summer 2020	31
Hysterothylacium deardorffoverstreetorum	(-)	(-)	(-)	(-)	(-)	(-)	63 fish Summer 2019, 2020, 2021	63
Mixed infestation	3 fish	(-)	1 fish	5 fish	(-)	13 fish	9 fish	31

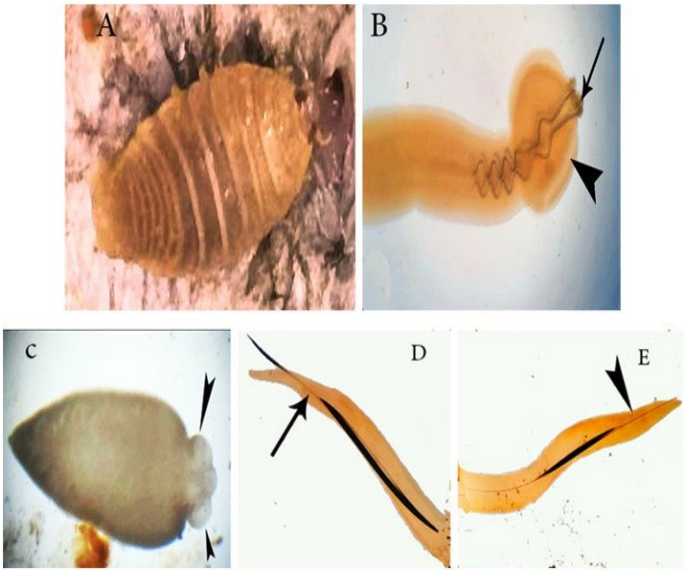

Fig. 1. Isolated parasites from marine fish demonstrated (A): *Isopoda* sp. (*Ceratothoa oestroides*) characterized by rigid, segmented exoskeleton with two pairs of antennae, seven pairs of jointed limbs on the thorax, and five pairs of branching appendages on the abdomen. (B) *Trypanorhyncha* larva (*Callitetrarhynchus gracilis*) stained with acetic carmine. Anterior part showing the characteristic bothria (arrow head), and hooked tentacles (arrow) (C) *Tetraphyllidean* larvae showing the evaginated scolex with four bothridia (D and E) Posterior and anterior ends of *Hysterothylacium deardorffoverstreetorum* respectively.

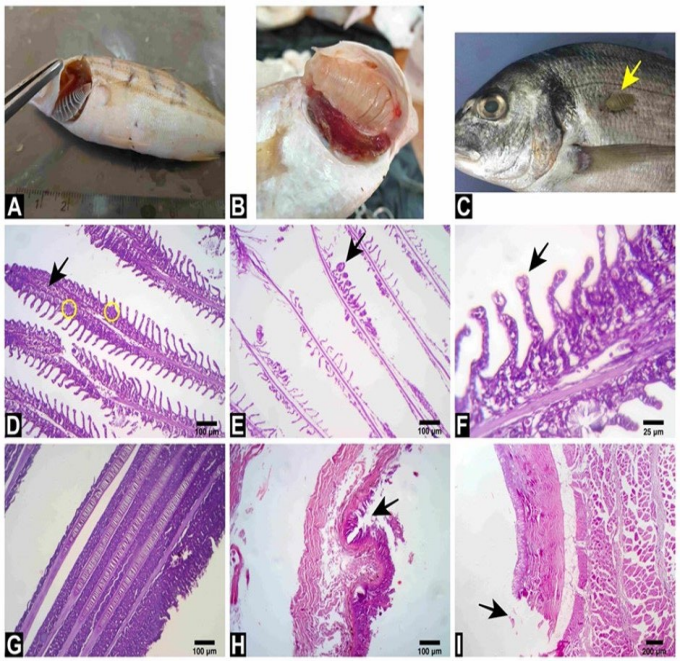

Fig. 2. Marine fish infested with *Isopoda* sp. (*Ceratothoa oestroides*) showing: (A and B) Parasite attached to the gills of *Pomadasys incisus* fish causing bulging of the overlying operculum causing its bulging. The gills are congested and excessive mucus secretion is evident (C) The parasite is attached to the skin (yellow arrow) causing descaling and ulceration of the skin at the attachment site. Photomicrographs of gills and skin of the infested gills showing: (D) Congestion of the central venous sinuses (black arrow), basophilic rounded colonies suggestive of epitheliocystis (yellow circle) with fusion of some secondary lamellae. (E) Massive destruction of the secondary lamellae accompanied by clubbing of the few remaining ones (black arrow). (F) Telangiectasis (black arrow) and partial fusion of the secondary lamellae (G) Gills Marked fusion of the primary lamellae. (H) Skin showing epidermal hyperplasia, erosions (black arrow) and ulcerations together with subepidermal edema and increased number of melano-macrophages. (I) Skin showing ulceration (black arrow) and hyalinization of the subepidermal muscular layer. Scale bar = 100 µm in D,G,E and H, 25 µm in F and 200 µm in I.

pale and anemic. Ulcerations and erosions were the main findings at site of attachment of the parasite to skin and heavy infestations were often accompanied by loss of scales (Fig 2C).

Microscopically, the infested gills showed a magnitude of changes, with the most common alterations being destruction of some secondary lamellar filaments and hyperplasia of the gill epithelium with partial fusion of others. Massive destruction of the secondary lamellae was often accompanied by clubbing of the remaining ones. The primary lamellae showed disorientation, bending and occasional fusion. The central venous sinuses were congested, and telangiectasis of the secondary lamellae and hemorrhages were evident. In some instances, basophilic rounded colonies suggestive of epitheliocystis were observed in the infested gills. The skin lesions consisted mainly of erosions and ulceration of the epidermal layer usually accompanied by goblet cells hyperplasia, hyperplastic epidermal borders, subepidermal edema and inflammatory cells infiltration. In some cases, deep sub-epidermal muscular layers showed hyalinization, necrosis, calcification and intermuscular hemorrhages and fibrosis (Fig 2D-I).

Trypanorenchea larvae (*Callitetrarhynchus gracilis*)

Grossly, the main findings were distension of the abdominal cavity. The plerocerci were represented as yellowish white nodules in the peritoneal cavity mostly attached to the liver surface and the internal organs causing variable degrees of adhesion (Fig. 3A and B). Severe congestion, edema and sometimes hemorrhage in most of internal organs (heart, spleen, liver) and mesentery were noticed.

Microscopically, the encysted plerocerci were mainly found in the peritoneal cavity sometimes attached to the liver surface. Rarely the larvae were observed in the stomach or intestinal wall. Some plerocerci were viable and others were degenerated. Sections of the encysted non-degenerated pleroceri show the typical tetrarhyncheal apparatus with the four bothria, tentacles and hooks. The characteristic basophilic calcareous corpuscles were evident in the larval parenchyma. The migration of the larvae throughout the viscera was indicated by the migratory tracks observed mainly in the liver and represented by hemorrhages, fibrin deposition and leucocytic infiltrations. Hepatic parenchyma also showed vacuolation and individualization of hepatocytes sometimes with pyknotic nuclei denoting hepatic necrosis. Notable melano-macrophage aggregations were observed in the liver and spleen (Fig. 3C-I).

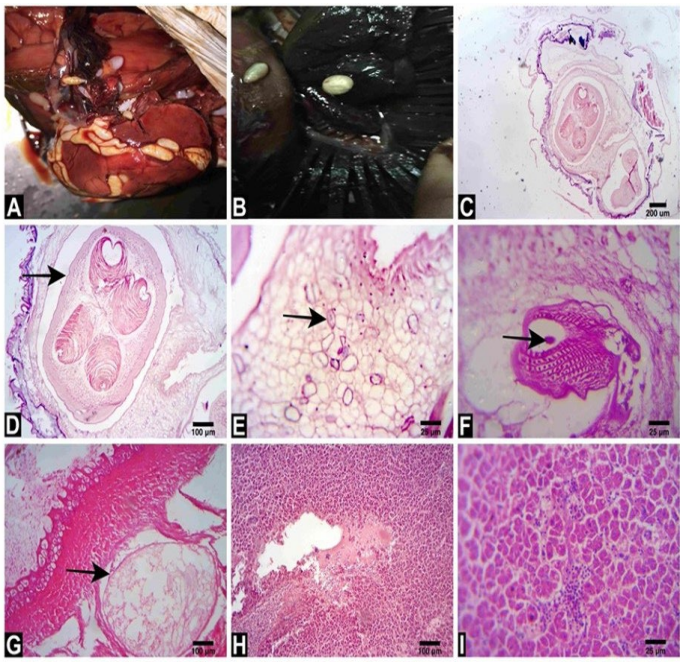

Fig. 3. Marine fish infested with *Callitetrarhynchus gracilis* showing: (A and B) Encysted plerocerci distribution in the peritoneal cavity and on the gills of infested fish. (C) photomicrograph of a section of a plerocercoid in the peritoneal cavity with calcification of the outer capsule. (D) A higher magnification of the previous plerocercoid showing the anterior end of the larva (black arrow) with four tentacles. (E) A higher magnification of the previous photomicrograph showing basophilic calcareous corpuscles (black arrow) in the larval parenchyma. (F) A high magnification of another plerocercus showing the details of the tentacle with the hooks and the retractor muscle (black arrow). (G) photomicrograph of a degenerated cyst (black arrow) in the stomach wall surrounded by a fibrous connective tissue reaction.(H) photomicrograph of liver showing a migratory track represented by hemorrhage, fibrin and leukocyte cells infiltration.(I) photomicrograph of liver showing congestion, vacuolation and individualization of hepatocytes, some of which show pyknotic nuclei. Scale bar = 200 µm in C, 100 µm in D,G and H and 25 µm in E,F and I.

Tetraphyllidean larvae

Macroscopically, numerous larvae filled cysts were embedded in tiny nodules with abundant fibrosis, producing visceral adhesions which is clear in few infested fish and hardly declared in others. Microscopically, the fish infested with *Tetraphyllidean* larvae showed degenerated larval cysts in the mesenteric cavity (Fig. 4A). The cysts frequently exhibited a calcified outer wall. The intestine showed degeneration and necrosis of the intestinal villi, and sloughed enterocytes were evident in the intestinal lumen. The intestinal submucosa showed marked hyperemia, slight edema, and prominent increase in the eosinophilic granular cells. The liver and spleen showed increased activity of the melano-macrophage centers. (Fig. 4B-D).

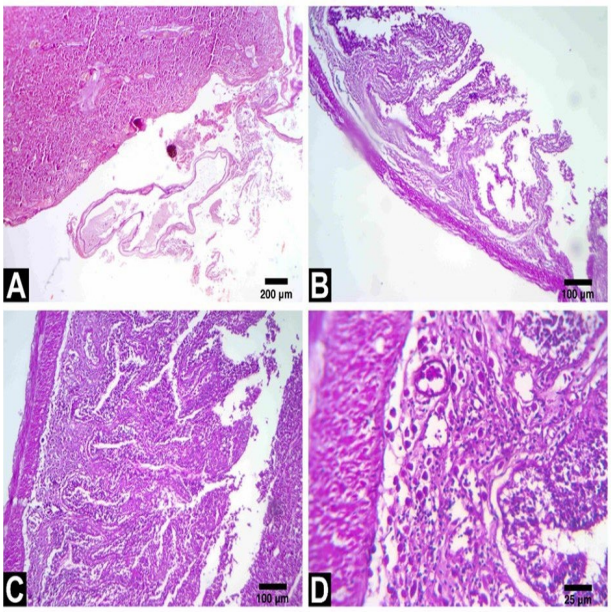

Fig. 4. Photomicrographs of the microscopic pathologic effects of *Tetraphyllidean* larvae infestation in marine fish showing A) A degenerated larval cyst in the mesenteric cavity close to the liver which shows increased number of melano-macrophage centers. B) A cross section of the intestine showing marked necrosis of the intestinal villi, severe loss of the intestinal epithelium and an increase in the eosinophilic granular cells in the submucosa. C) A section of the intestine showing partial fusion of the intestinal villi, necrosis and desquamation of the lining enterocytes which fill the intestinal lumen and pronounced hyperemia of the mucosal and submucosal blood vessels. D) A higher magnification of the intestine showing necrosis and loss of the intestinal epithelium, hyperemia of the submucosal blood vessels with slight edema. Scale bar = 200 µm in A, 100 µm in B and C and 25 µm in D.

Hysterothylacium deardorffoverstreetorum

Microscopical findings in fish infested with hysterothylacium mainly exhibited pathologic lesions in the digestive tract ranging from moderate irritation of the intestinal mucosa as represented by goblet cell hyperplasia, increased mucus secretion in the intestinal lumen and hyperemic mucosa and submucosa (Fig. 5A and B) to severe devitalization of the intestinal mucosa as represented by denuded and stunted villi, presence of sloughed villi in the lumen and intense lymphocytic infiltration in the submucosa. The spleen showed increased number of the melano-macrophage centers, which was also evident in the liver (Fig.5 C and D). In addition, the liver was frequently congested and media of blood vessels were commonly hypertrophied.

Discussion

In this study we recorded the prevalence of four different parasitic species in 400 marine fish randomly collected from the Egyptian coasts during the period between winter 2019 to summer 2021 and described the pathologic lesions induced by these infestations. The identified parasites were a crustacean isopod; *Ceratothoa oestroides*, two larval cestodes; *Callitetrarhynchus gracilis* and *Tetraphyllidean* larvae, and a nematode; *Hysterothylacium deardorffoverstreetorum*.

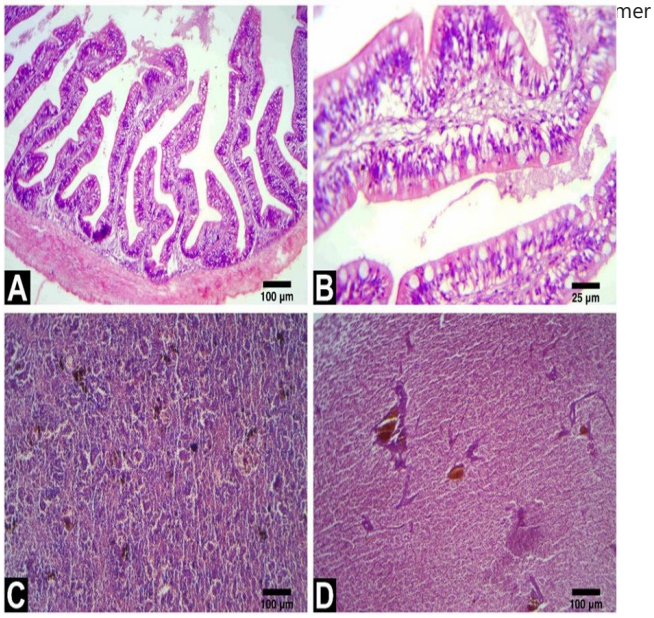

Fig. 5. Photomicrographs of the microscopic pathologic effects of *Hysterothylacium deardorffoverstreetorum* infestation in marine fish showing A) Goblet cell hyperplasia in the intestinal mucosa, increased mucus secretion in the lumen and partial hyalinization of the muscular layer. B) A higher magnification of the previous photo showing goblet cell hyperplasia and increased mucus secretion. C and D) are showing increased number of melano-macrophage centers in the spleen and liver respectively. Scale bar = 100 µm in A, C and D and 25 µm in B.

of 2020 by *Ceratothoa oestroides*. An increase in *Isopoda* prevalence was also reported by Helal and Yousef (2018) and Fahmy (2020) in the lake Qarun, and Mahmoud *et al.* (2019) in Mediterranean sea fries. We found the parasite either attached to the gills and protruding below the operculum or attached to the skin causing loss of scales at site of attachment. The main gill lesions microscopically were fusion of the primary and secondary lamellae, hyperplasia of the gills' epithelium, congestion of the central venous sinuses and telangiactasia of the secondary lamellae. The skin showed hyperplasia, erosions and ulcerations at site of attachment of the parasite. These findings support descriptions from previous studies (Rameshkumar and Ravichandran 2014; Mladineo *et al.*, 2020) and is mostly attributed to the feeding mechanism of the parasite and the irritation induced by its attachment. An interesting finding was the frequent observation of Epitheliocystis colonies in the gills infested with *Ceratothoa oestroides* and it's unclear whether the isopod might play a direct role in transmission of epitheliocystis or indirectly facilitate infection through exhausting the fish immunity. This is in line with findings reported by Eissa *et al.* (2015) who documented 50% prevalence for *Isopoda* parasites in marine fish collected from Suez and Ismailia and highlighted its association with Pseudomonas aeruginosa infection and in accordance with Rameshkumar *et al.*, 2013) who found higher bacterial loads at site of attachment of *Isopoda* and (Mladineo *et al.*, 2020) who described a bacterial biofilm covering the mouthparts of *Ceratothoa oestroides*, although Epitheliocystis was not particularly mentioned in these studies.

The second most prevalent parasite in our study was *Hysterothylacium deardorffoverstreetorum* with a prevalence rate 31.98%. The parasite was only isolated from *Caranx crysos*, and the main microscopic lesions inflicted by this parasite were mainly observed in the digestive tract and covered a wide spectrum from goblet cell hyperplasia and increased mucus secretion indicating irritation of the mucosa to severe necrosis and sloughing of the intestinal villi. This matches the report of Hysterothylacium larvae isolated mainly from the intestine of infested fish in the Red sea (Khalifa *et al.*, 2019).

We reported here the isolation and identification of the cestodal trypanorhynchid larva; *Callitetrarhynchus gracilis* from marine fish. We observed the larval blastocyst in the mesenteric cavity, on the liver surface and on the gills. Microscopically, viable pleroceri showed characteristic hooked tentacles at the anterior end of the parasite. Degenerated cysts were frequently encountered microscopically as well. Migration of the larvae through the internal organs was indicated by the presence of migratory tracks in the viscera and increased activity of the melanomacrophage centers in the liver and spleen. These findings broadly support the work of other studies (Abdou and Palm 2008; Mahmoud *et al.*, 2015; Abdelsalam *et al.*, 2016)

The least prevalent parasites isolated in our study were *Tetraphyllidean* larvae. The main pathologic effect of *Tetraphyllidean* larvae observed in this study was in the intestinal tract probably due to attachment of

the parasite to the intestinal wall although degenerated cysts were also detected in the mesenteric cavity. Our description of the microscopic lesions induced by *Tetraphyllidean* larvae is in partial accordance with those described by Santoro *et al*. (2013).

Conclusion

This study provided an insight on four prevalent marine fish parasites isolated from different Egyptian coasts between winter 2019 and summer 2021 and documented the pathologic picture associated with their infestation in the host fish emphasizing the continuous need for improving our marine fish environment and applying the most suitable and secure parasite control methods to spare our marine fish industry the unnecessary effects of these infestations.

Conflict of interest

The authors declare no competing interests.

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