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Histopathological alterations and condition factor deterioration accompanied by isopod infestation in *Tilapia zilli*, *Mugil capito* and *Solea aegyptiaca* from Lake Qaroun

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ABSTRACT

This study was carried out to identify isopod species implicated in the outbreak that had affected commercially important fishes of Lake Qaroun. The study also aims to determine its deleterious effects on infested fish and its possible role in the severe drop in lake fisheries production. *Renocila thresherorum* (Crustacea: Isopoda: Cymothoidae) was the only isopod species isolated from the fishes in the lake. The overall prevalence was 38% as 37.14, 25.7 and 53.3% of the examined *Tilapia zilli* (Gervais, 1848), *Mugil capito* (Cuvier, 1829) and *Solea aegyptiaca* (Chabanaud, 1927), respectively, they were found to be infested; while the recorded intensity was 1.23 for *T. zilli* and 1 for both *M. capito* and *S. aegyptiaca*. The main clinical sign noticed was a unilateral or bilateral bulged operculum, with the presence of a large sized isopod female which reached up to 23 mm in length in gill chamber, pale atrophied and eroded gill filament. The condition factor of infested fish significantly decreased in comparison with that of non-infested fish. Various degrees of degenerative pathological lesions including destruction, detachment, hyperplasia and fusion of the primary and secondary gill lamellae were observed in infested fish gills. The current research proved the deleterious effect of *R. thresherorum* on Lake Qaroun fishes based on clinical finding, condition factor calculation and histopathological examination.

Introduction

Isopods are dorsoventrally compressed parasitic crustaceans that infest a wide range of marine and freshwater fishes and invertebrates (Bruce, 2004; Ravichandran et al., 2010). The term isopod is derived from the great similarity between their legs (iso means identical or similar and pods means legs). Out of 95 isopod families, constituting of more than 5700 different species, about 450 species are associated with fish infestation (Williams and Bunkley-Williams, 1996; Smit et al., 2014). Cymothoidae is a large isopod family that has 40 genera and about 380 species (Ahyong et al., 2011); they are obligate parasites that affect a wide variety of fish species. Isopods infestation is a direct cause of huge economic losses in commercially important fish species including body weight loss, direct mortality, particularly in small fish, and loss of fecundity (Rajkumar et al. 2005; Youssef et al. 2014; Chinabut, 2002; Praveenraj et al., 2017; Kumar et al., 2012).

Lake Qaroun is an enclosed inland lake located in Fayoum governorate, Egypt, it occupies about 23,000 ha. The Lake's salinity ranges between 30 and 40 ‰ in winter and 38–42‰ in summer season (Azab et al., 2015; Abdelaziz et al., 2017). Lake fisheries' production severely dropped from 4518 ton in 2014 to 1124 ton in 2015 (GAFRD, 2016 & GAFRD (2017)); this huge negative change was attributed to isopod invasion of affecting commercially important fish, including *Tilapia zilli*, *Mugil capito*, *Solea aegyptiaca* and *Dicentrarchus labrax*.

The present study aims to identify the parasitic isopods infesting Lake Qaroun fishes and to determine their role in disease pathology and their effects on fish health status represented in condition factor.

Materials and methods

Study area: fish samples were collected from Lake Qaroun located in Fayoum governorate, central Egypt. The lake lies between latitudes 30°.41′–30°.82′ east and longitudes 29°.44′ - 29°.51′ north.

Fish specimens: A total number of 100 fish were collected alive as a sample [35 *Tilapia zilli* (family: Cichlidae), 35 *Mugil capito*

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(family: Mugilidae) and 30 *Solea aegyptiaca* (family: Soleidae)]. Samples were collected during May 2015 by gill nets and trawling (mesh size of used net was 22 mm from knot to knot). After that they were transported immediately to fish diseases lab, Shakshuk station, National Institute of Oceanography and Fishery (NIOF).

Collected specimens ranged between 10 and 18, 14.5–16 and 14–17 cm in total length, 8.3–10.1, 29.7–35.3 and 29–37 g in weight for *T. zilli*, *M. capito* and *S. aegyptiaca,* respectively Table 1.

Clinical examination: was performed as described by Noga (2010) for the determination of any external abnormalities on infested fish.

Condition factor (K): Ten infested and 10 non-infested fish from each species (except *M. capito* 9 infest fish) were used for condition factor calculation using the following formula: $K = 100\ W/L^3$ (Sutton et al., 2000), in which W is fish weight in grams and L is fish total length in centimeter.

Parasitological examination: isopod samples were recovered from the branchial cavity of infested fish then fixed in AFA (alcohol formalin acetic acid) as described by Woodland, (2006). Fixed isopods were measured and classified based on their morphological features as recorded by Williams and Williams (1980), Brusca (1981) and Bruce (2004).

Table 1
Body weight and total length of non-infested and *Renocila thresherorum* infested fish.

Fish species	Item	Non-Infested	Infested	No. of Samples
Tilapia zilli	Weight	12.95 ± 2.12	14.68 ± 2.55	10
	Length	9.16 ± 0.61	9.26 ± 0.6	10
Mugil capito	Weight	32.39 ± 2.17	31.67 ± 2.14	9
	Length	15.37 ± 0.62	14.47 ± 0.9	9
Solea aegyptiaca	Weight	34.07 ± 2.49	31.71 ± 2.43	10
	Length	15.35 ± 0.74	16.64 ± 0.97	10

Values are mean ± standard deviation

Fig. 1. (a) *Tilapia zilli* infested with *Renocila thresherorum* (white arrow) with unilateral bulged operculum. (b) *T. zilli* with bilateral bulged operculum (infestation of both gill chambers). (c) *T. zilli* with marked gill cover erosions (arrow) and shortening of some primary gill lamellae (star). (d) *Solea aegyptiaca* infested with *R. thresherorum* (arrow) in dorsal gill chamber. (e) *Mugil capito* showing necrosis in primary gill lamellae (arrow) with presence of large *R. thresherorum* female in branchial chamber. (f) *M. capito* infested with *R. thresherorum* male (arrow) in branchial chamber.

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Overall prevalence and intensity: were determined using Q3 software as described by Rozsa et al., (2000).

Histopathological examination: infested fish gills were dissected, immediately fixed in 10% neutral buffered formalin, dehydrated in ascending grade ethyl alcohol, sectioned to 5 μm and stained with H & E. After that they were examined and photographed using Optika microscope with digital camera (Optika, Italy), as mentioned by Roberts (2012).

Statistical analysis: The results of condition factor calculations for infested and non-infested fish were expressed as mean ± stan dard deviation. Values were compared by paired samples *t*-test using MedCalc for windows, version 17.2.2, (MedCalc Software, bvba, Ostend, Belgium, https://www.medcalc.org; 2017).

Results

Clinical examination

Clinical investigation of diseased fish indicated unilateral bulged operculum of all infested fish except in some cases of *T. zilli*, it became bilateral (both gill chambers were infested). All collected isopods were recovered from gill chamber, pale atrophied gills with detachment of primary gill lamellae even gill arch cartilage appeared in severely affected fish. In a few cases, marked gill cover erosions were present. In *S. aegyptiaca,* the parasite was recovered only from the upper gill chamber (at the dorsal body side) (Fig. 1).

Table 2
Condition factor (K) of non-infested and infested fish with *Renocila thresherorum*.

Fish species	Condition factor (K)			
	Non-Infested	Infested	P	No. of Samples
Tilapia zilli	1.9^{a} ± 0.13	1.68^{b} ± 0.19	0.018	10
Mugil capito	1.06^{a} ± 0.14	0.87^{b} ± 0.08	0.014	9
Solea aegyptiaca	0.95^{a} ± 0.11	0.7^{b} ± 0.07	0.0025	10

Values are mean ± standard deviation, different superscription letters means significant change.

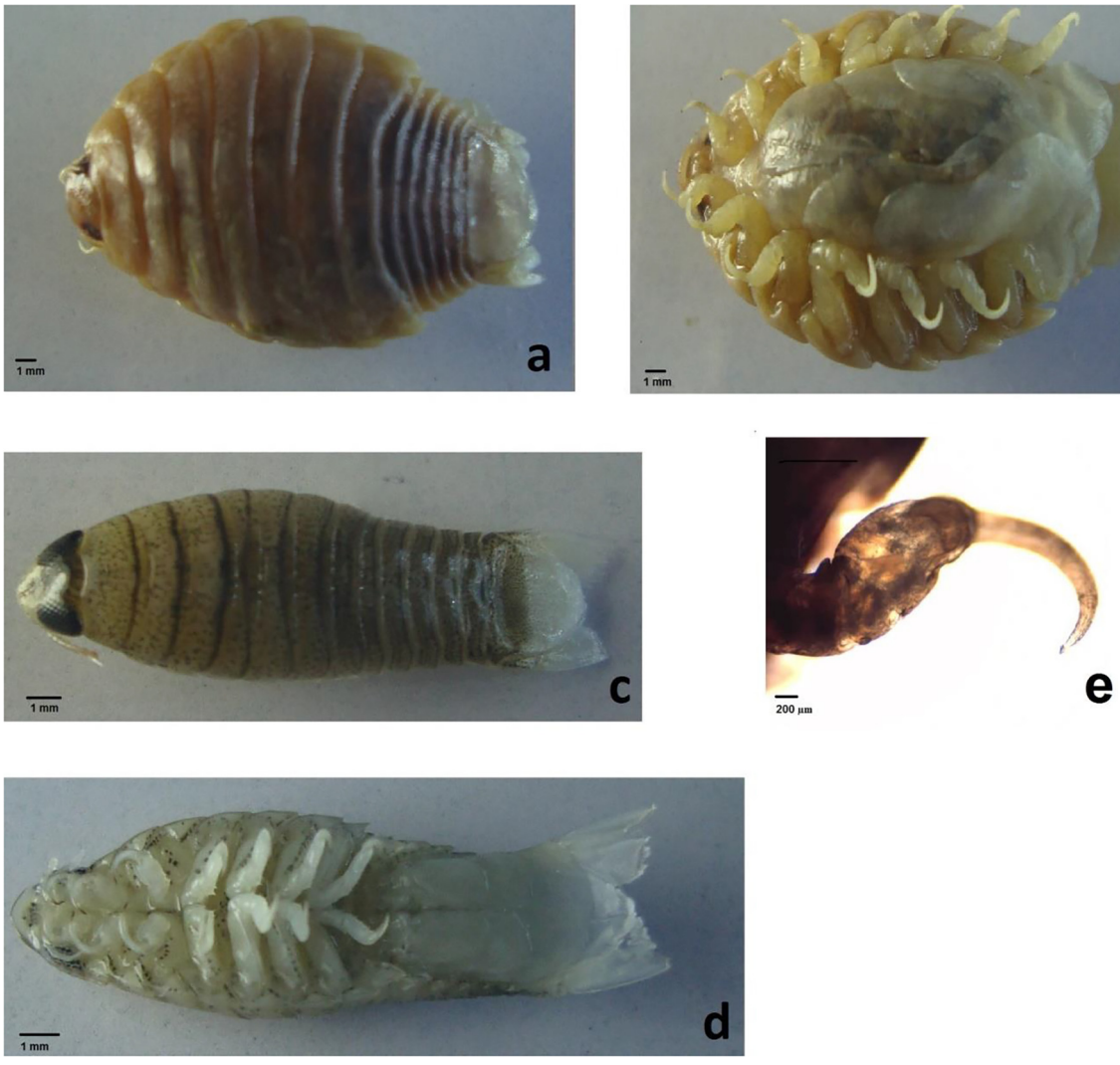

Fig. 2. (a) *Renocila thresherorum* female dorsal view, large flattened dark brown. (b) *R. thresherorum* female ventral view, seven pairs of pereopod (legs), large brood pouch (marsupium) contain pullus stage and non-hatched eggs. (c) *R. thresherorum* male dorsal view, thin, elongated with dark scattered chromatophores. (d) *R. thresherorum* male ventral view with seven pair of legs. (e) Sharp hook (dactyli) about 1 mm in length, magnification power, X = 40.μ.

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Condition factor

Condition factor calculation indicated a significant decrease in isopoda infested fish body condition for all studied fish species when compared with that of the non-infested fish of similar length (Table 2).

Parasitological examination

The parasitological examination revealed the presence of *Renocila thresherorum* (Crustacea: Isopoda: Cymotidae), female is dark brown in color, has a flattened body, 16–23 mm in body length, 0.8–11.5 mm in width at 5th pereon, it weighed between 0.45 and 1.1 g. The female body consists of small cephalon, it carries 2 large dark compound eyes, seven thick pereon segments and 5 thin pleon ones. It has 7 pairs of legs (pereopod), the first 3 pairs of legs are directed anteriorly, while the last 4 pairs are directed posteriorly each ending with a sharp hook (dactyli) of about 1 mm for attachment and fixation with gill tissue (Fig. 2, e). The female has large brood pouch (marsupium) in ventral surface filled with spherical eggs, larvae (pullus) was found in some gravid females, marsupium formed from overlapping oostegites that arose from the bases of pereopods. Antenna and antennule are equal in length and each contains 8 segments (Fig. 2, a & b).

The male is elongated and thin in comparison with the female, it is also lighter in color than the latter. It measures 17 mm in body length, 6.5 mm in width at 5th pereon and it has the same other morphological features of the female (Fig. 2, c & d).

Larvae or pullus (pre-manca stage) were recovered from brood pouch, they ranged between 2225 to 2578 μm in length and 610 to 780 μm in width at pereon. Pullus has six pairs of legs and visible yellow yolk globules at the pereon region (Fig. 3, a–c).

The eggs are spherical, yellow to brown in color, measuring 1013–1028 × 981–983 μm in diameter, the large brood pouch carries 60–87 eggs (Fig. 3, d).

Overall prevalence and intensity

The overall prevalence of *R. thresherorum* infestation in lake fishes was 38%, while the recorded prevalence for *T. zilli*, *M. capito* and *S. aegyptiaca* is 37.14, 25.7 and 53.3%, respectively (Table 3). The intensity (number of collected parasites/number of infested fish) was 1 for *M. capito* and *S. aegyptiaca* while it was 1.23 for *T. zilli* (16 parasites were recovered from 13 infested fish as 3 fishes had bilateral infestation).

Histopathological examination

Histopathological examination of affected gill tissue from examined fish specimens revealed the presence of several degenerative changes with various degrees. The primary gill lamellae were curved in mild cases (Fig. 4, c), while in severe cases most of the primary gill filament were detached (Fig. 4, e–h). Regarding the secondary gill lamellae, the pathological changes in the mild case were hyperplasia, curling and fusion (Fig. 4, c–h). In the severe case, loss of normal tissue architecture, complete erosion of

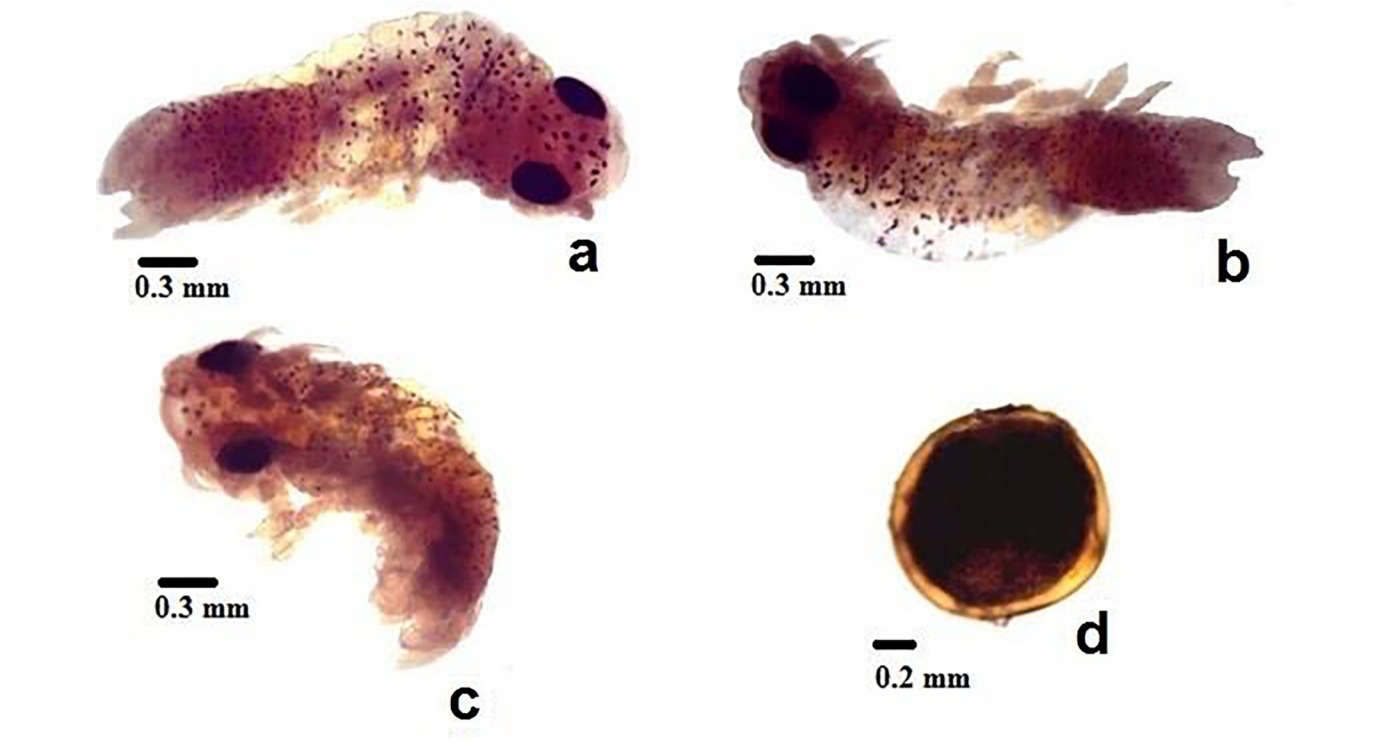

Fig. 3. (a & b & c) *Renocila thresherorum* larvae (pullus or pre-manca stage) recovered from brood pouch with obvious large compound eyes, six pair of legs and visible yellow Yolk globules in pereon. (d) *R. thresherorum* spherical egg about 1 mm in diameter, magnification power, X = 40.

Table 3
The overall prevalence and intensity of Renocila thresherorum infesting *Tilapia zilli, Mugil capito, Solea aegyptiaca.*

Fish species	No. of examined fish	No. of infested fish	Infestation %	Intensity (No. of parasites per infested fish
T. zilli	35	13	37.14	(16/13) 1.23
M. capito	35	9	25.7	(9/9) 1
S. aegyptiaca	30	16	53.3	(16/16) 1
Total	100	38	38	1.08

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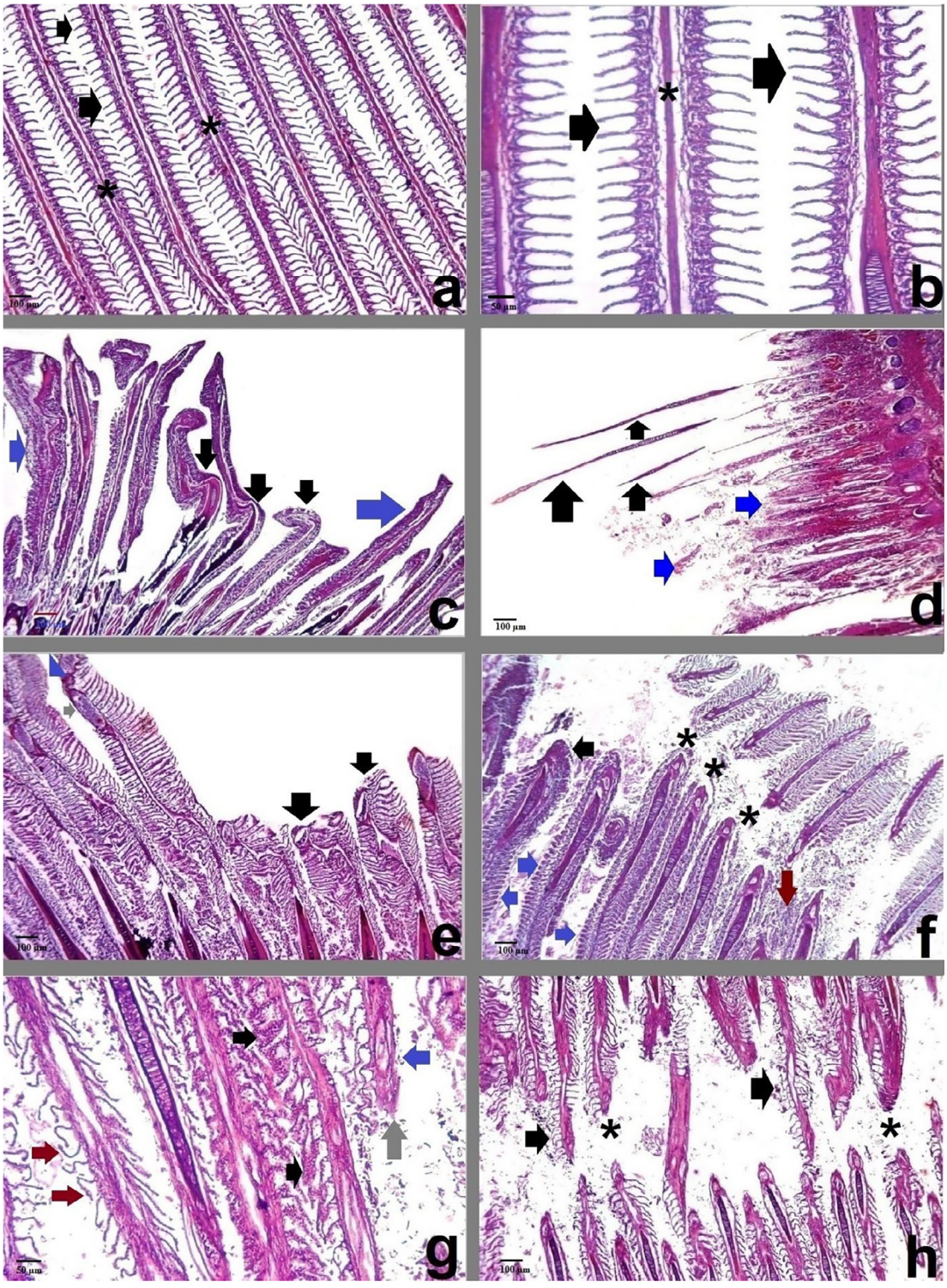

Fig. 4. (a & b) Normal gill architecture of *Tilapia zilli*, primary gill lamellae (star), secondary gill lamellae (arrow), power a = 40 X and b = 100 X. (c) Infested *T. zilli* with curved primary gill lamellae (black arrow) showing hyperplasia and fusion of secondary gill lamellae (blue arrow) 40 X. (d) Infested *T. zilli* gills showing massive destruction of primary gill lamellae, complete loss of secondary gill lamellae even appearance of cartilaginous core of the primary gill filament (black arrow), necrotic tissue and tissue debris (blue arrow), 40 X. (e) infested *Mugil capito* gills with shortening and removal of large area from primary gill lamellae (black arrow), secondary lamellae fusion (blue arrow) and complete sloughing of secondary gill lamellae (gray arrow) 40 X. (f) infested *M. capito* gills with complete destruction and degeneration of cartilage core supporting some primary gill filaments (stars), fusion of secondary gill lamellae (blue arrow), hyperplasia (black arrow) and presence of destructed tissue debris (brown arrow) 40 X. (g) *Solea aegyptiaca* gills, detachment of some primary gill lamellae (gray arrow), complete sloughing of secondary gill lamellae (blue arrow), hyperplasia of secondary gill lamellae epithelium (black arrow) and curling in secondary gill lamellae (brown arrow) 100 X. (h) *S. aegyptiaca* gills, detached parts from primary gill lamellae (star), curling in secondary gill lamellae (black arrow) 40 X.

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secondary gill lamellae even appearance of cartilaginous core of the primary gill filament, were registered (Fig. 4, d) along with the presence of tissue debris from sloughed necrotic gill tissue (Fig. 4, d & f).

Discussion

The results of the present study suggests that the severe drop in lake fisheries production might be attributed to isopod infestation, as the lake had produced 4518 tons in 2014 before the isopod outbreak that caused a severe drop to 1124 tons in 2015 after a flare up of infestation. Isopod infestation can affect fish through different ways including lowering the growth rate and decreasing fecundity; it is a direct reason to high fish mortality especially for fry and fingerlings. Diseased fish also become highly susceptible to bacterial infection.

Regarding the clinical signs, bulged operculum was a result of the presence of large sized isopods (16–23 mm) which prevent complete closure of gill cover. Gill filaments of infested fish turned pale, atrophied and eroded, these signs were mainly attributed to the pressure atrophy that is induced by the presence of the parasite. The parasite was recovered only from the dorsal gill chamber of *S. aegyptiaca* that can be referred to frequent contact between the lower side and pond floor (lake bottom).

Decreased condition factors vouched the negative impact of isopoda infestation on affected fish. A decrease in (K) almost came as a result of inability to feed normally. The feeding of wild fish depends on continuous pumping of the water current through gills during respiration, this is accompanied with water filtration using gill raker that collects nutrients including algae. The previously mentioned step is then followed by the swallowing of collected food particles to the fish's stomach, the efficacy of this process is markedly decreased by imperfect closure of gill cover resulting in under feeding followed by emaciation that manifested in decreasing the condition factor. Ravichandran et al., (2009) referred the stunted growth of isopod infested fish to nutritional drain by the parasite. Ravi and Rajkumar, (2007) also recorded decreased growth of *Oxyurichthys microlepis* infested with *Cymthoa indica.*

Based on the morphological features, the present study identified *R. thresherorum* as the only isopod species recovered from infested *T. zilli*, *M. capito* and *S. aegyptiaca* specimens that were collected from Lake Qaroun. Mahmoud et al., (2016) and Shaheen et al., (2017) also identified the same species from infested fishes in Egypt. To our knowledge, isopod is a new invasive fish parasite in Lake Qaroun as it was not recorded before 2014. The method by which such parasite was transported to the lake was not clear. However, we suggest that it might have been through infested marine fish fries that have been transported to the lake especially from endemic areas in the Red Sea. The research done by Youssef et al., (2014) supports this theory as they isolated *R. thresherorum* from infested marine fish from the Suez Canal area at Ismailia Province where there is a fry collection center related to the General Authority for Fish Resources supplying Lake Qaroun with marine fish fry.

The current observations linked the intensity of the parasite's prevalence in different fish species to the swimming speed of infested fish; i.e. *M. capito* has a forked caudal fin, so it is a fast swimming fish and can avoid isopod attachment, while tilapia has truncated tail and has less swimming speed than mugil so it can be easily attacked by isopods. *S. aegyptiaca* is a slow swimming fish and lies over the lake bottom which explains the highest infestation rate. Moreover, the suitable environmental conditions of high salinity and abundance of food, represented in fish, give rise to the overall high prevalence (38%).

The recorded histopathological changes can be attributed to the presence of large isopod (*R. thresherorum*) in gill chamber fixed to gill filament with sharp dactyli that apply great pressure on first gill filament, in the mild case it leads to deformity (curved primary lamellae). While in the severe case, the parasite prevents blood supply to reach the majority of gill filament leading to atrophy and necrosis followed by the sloughing of the affected part. These findings are in complete agreement with the work of Kabata (1985); Rameshkumar and Ravichandran (2014) and Thamban et al. (2016) who linked gill tissue destruction associated with this infestation to the pressure exerted by the isopod body. Moreover, Kumar et al. (2017) considered Cymothoids one of the largest fish parasites responsible for obvious tissue destruction While Boxshall (2005) referred atrophy to feeding and attachment of Cymothoid to gill tissue.

The present study proved the deleterious effects of *R. thresherorum* on Lake Qaroun fishes based on the clinical findings, condition factors and histopathological examinations. *R. thresherorum* negatively affects fish through different ways including vigorous feeding on fish serum rich in nutrients that deprives fish from essential nutritional elements, together with the inability of fish to feed normally (non-perfect closure of operculum and so decrease the passing water current that is rich in nutrients). Firm attachment of the parasite with gills' tissue using 14 sharp dactyli cause constant irritation to fish, it also results in wounds and erosions which act as a porter for the entry of many bacterial pathogens and are responsible for many histopathological alterations. Finally, based on the obtained calculations (the mean weight of both isopod female and infested fish), this parasite represents 2.5% of *M. capito*, *S. aegyptiaca* and 6% of *T. zilli* body weight so it is considered one of the largest parasites in relation to their host body weight.

Conclusion

The current study associated the severe drop in Lake Qaroun fishery production to the isopod infestation outbreak affecting its fishes. *Renocila thresherorum* isopod is mainly responsible for different pathological lesions including atrophy and necrosis in gill filaments, along with the decreased condition factor of the affected commercially important lake fishes.

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