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A cross-sectional Analysis and Immunological Study of Parasitic Infections in *Solea aegyptiaca* and *Tilapia zillii* from Qarun and Wadi El-Rayan Lakes

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Abstract

NOWADAYS, Qarun and Wadi El-Rayan lakes are exposed to adverse ecological changes that have led to the loss of fish stocks. In this study, we recorded the most common parasitic infestations in these lakes regarding bad water quality parameters and the associated parasitological and pathological effects. A total of 274 fish species were categorized into 205 *Tilapia zillii,* and 69 *Solea aegyptiaca* were randomly collected from Wadi El Rayan lakes; private fish farms and Shakshouk area of Lake Qarun in Fayoum governorate, Egypt. Total prevalence of parasitic infection was 33.9 %. All of the examined fish were positive for one or two mixed parasitic infections. *Trichodina* spp., *Gyrodactylus* spp., *Cichlidogyrus tilapiae, Centrocestus formosanus* encysted metacercaria and *Livoneca redmanii* were detected in 4.3%, 11.2 %, 13.6 %, 14.6% and 4.3% of infected fishes respectively. The immunological response of fish to parasitism was detected via the upregulation in levels of *TNFα* and *IL-1β* genes with significant differences (P =0.000). The obtained physicochemical water parameter results from studied places indicate that ammonia, nitrite, nitrate, and phosphate concentrations were above the specified permissible limits. The histopathological lesions associated with parasitic infestation described severe hyperplasia, degeneration and necrosis of gill lamellae; congestion of branchial blood vessels, associated with the presence of *Trichodina* spp., and encysted *C. formosanus* in gills. These all highlight how important it is to comprehend how these parasites can affect fish health, productivity and the aquaculture industry. However, the presence of these parasites in fish-rearing water requires more financial and management efforts.

Keywords: Ecological changes, *TNFα*, Physicochemical analysis, Histopathological, Fayoum.

Introduction

Egypt is a leading African country in the aquaculture industry, accounting for around 1.8 million tons of total production [1]. Fayoum governorate shares in fish production via Lake Qarun and Wadi El-Rayan lakes inland capture fisheries. Qarun Lake is one of the most important fisheries in Egypt, it is a closed saline basin located in the northern-western part of El Fayoum governate [2]. The Lake's main sources of water are agriculture drainage and sewage wastewater from two main drains, El-Batts and El-

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Wadi [3]. The total catch of Qarun Lake reached a maximum of 4518 tons in 2014 and from 2015 onward, the total catch decreased substantially to reach 832 tons in 2018 [4], as no fish fries were released to the lake, and consequently, all inhabitants fish as solea, tilapia fish and even shrimp catch were dramatically decreased [5]. Nowadays, the lake is exposed to adverse ecological changes in the form of increased water salinity, the disappearance of some inhabitant fish species, and an increase in bacterial and parasitic infection rates, leading to the loss of fish stocks. [6]. Understanding the parasite burden in these species is crucial for assessing the risk to Egyptian consumers and for developing targeted interventions [7].

One of the biggest obstacles facing Qarun and Wadi El-Rayan lakes is the drastic changes in the Physico-chemical water parameters; they are considered an important factor that aids in fish parasitism. Environmental pollution and the resulting reduced water quality are having its effect on marine resources, with the stress that brings on fish, the immune system is exhausted, and fish are more vulnerable to parasitic infections [8,9] Fish parasites are among the pathogens of concern in aquaculture [10,11,12]. *Trichodina* is one of the most common ectoparasitic ciliates present on the skin and gills of fish [13]. Heavy infestation with *Trichodina* can cause serious threats, particularly under cultural conditions as a consequence of tissue damage, causing serious economic losses [14]. *Cichlidogyrus*, *Gyrodactylus* and *Dactylogyrus* belong to one of the most monogenean species classes of fish parasites commonly found on fish gills and skin and may lead to significant fish mortality [15, 16].

Within fish-borne trematodes, the digenetic trematode *Centrocestus formosanus* causes pathological gill lesions and mortalities in various freshwater fish species [17,18]. The metacercariae may affect growth and survival, or cause skeletal fish deformity so that they lose their marketability as a food or ornamental product [19,20,21].

Since 2015, a heavy abundance of the *Cymothoidae* was reported in Lake Qarun, Cymothoid isopod was assumed they come from the Mediterranean Sea during fish fry transportation to the lake. It is an obligate crustacean ectoparasite, with a holoxenic life cycle that may extend from days to years, the larvae of parasitic isopods could infest various parts of the fish body such as the body surface, fins, inside the mouth, gills, or occasionally burrowing in special tunnels in the musculature of their host on which they feed and grow in size this lead to sever tissue damage, growth retardation, host behavioral abnormalities and high fish mortalities [22,23,24], consequently causing severe damage to the lake fish stock and disappearance of small fishes **[**25].

In fish, parasite infections can prompt major molecular alterations in the form of upregulation of fish immune gene expression [26]. Cytokines such as tumor necrosis factor (TNFα) and Interleukien-1β (IL-1β) are released by immune cells in response to a variety of pathogenic, parasitic, bacterial, and viral infections [27,28, 29].

The consequences of such parasitic infections extend beyond individual health, affecting growth rates, overall health, and economic performance of fish populations [30]. In light of these challenges, the primary objective of this research is to 1) Investigate the parasitological and histopathological effects of the isolated parasitic infestation in *Tilapia zillii* and *Solea aegyptiaca* fish. 2) Genetically identify and detect immune gene expression of infected fishes. 3) Emphasis on water quality parameters of selected localities. By examining the prevalence, pathological effects, and genetic characteristics of these infections, this study contributes to enhancing our understanding of the complex dynamics between parasitic pathogens and fish populations in aquaculture systems.

Material and Methods

Fish sampling and localities

A total of 274 fish species were categorized into 205 *Tilapia zillii* with an average body weight of 29± 2 g, total body length of 12.5±2 cm, and 69 *Solea aegyptiaca* with an average body weight of 30 ±5 g and total body length of 13±2 cm, were randomly collected from different areas in Fayoum governorate, Egypt during the studying period from October 2020 to June 2022. *T.zillii* were collected from Wadi El Rayan lakes (n= 157), and private fish farms of Lake Qarun (n= 48) while *S. aegyptiaca* species were only collected from the Shakshouk area, Lake Qarun. Fish specimens were sourced from distinct localities dedicated to the cultivation of their species as shown in Fig. 1. Fish were transported alive to the laboratory of the Parasitology Department at the Faculty of Veterinary Medicine, Cairo University, for further parasitological and histopathological examination.

Clinical investigation and Parasitological examination

All fishes were examined externally for any clinical abnormalities and presence of macroscopic ectoparasites, then smears from gills were prepared and examined under a light microscope (Olympus CX41 microscope, Japan) for detection of the common parasites (*monogeneans* sp.; *Centrocestus* sp.; *Trichodina* sp.) according to Salem et al. [28]. Moreover, muscle specimens were collected from the head, trunk, and tail regions. All the investigated specimens were dissected, mixed with drops of saline solution, compressed between two glass slides, and examined under a stereomicroscope (Olympus sz 61 microscopes, Japan) according to Mahdy et al. [27]. The body cavity of each fish was opened and examined by the naked eye for the presence of macroscopic parasites or abnormal lesions of the different organs. The viscera were removed, and each organ was placed in a Petri dish with saline solution and examined for the presence of cysts and parasites using the microscope. The intestinal tract of each fish was opened by scissors, left in normal saline solution and examined for visible parasites by using a hand lens. The specimens were thoroughly checked for parasitic isopoda infection in the body surface, fins, head, gills and oral cavities. The isopod parasites were removed from the fish and were fixed in 10% formalin and preserved in 70% alcohol according to Mahmoud et al. [23].

TNF-α1 and IL- 1β genes expression analysis using Quantitative real-time polymerase chain reaction (qRT-PCR)

Gill tissues (30 mg) (n=3) of infected fish with *Livoneca redmanii*, *C. formosanus* EMC and *Trichodina* were aseptically dissected and examined for gene expression analysis, control samples from gills of apparently uninfected fish were also examined. Total RNA was extracted using RNeasy Mini Kit (Qiagen, Germany) according to manufacturer instructions. The quality of extracted RNA samples was checked using a Nanodrop UV-Vis spectrophotometer (Thermo Fisher Scientific, US). The two gene primers used were tumor necrosis factor-α1 (*TNF-α1*) and interleukin-1β (*IL- 1β*) specific for *Tilapia* spp. as follows: *TNF-α1*, F (CCAGAAGCACTAAAGGCGAAGA), *TNF-α1*. R (CCTTGGCTTTGCTGCTGATC) according to Standen et al. [31] and, *IL-1β*-F (GCTGGAGAGTGCTGTGGAAGAACATATAG), *IL-1β*-R (CCTGGAGCATCATGGCGTG) according to Castro et al. [32]. The quantitative RT- PCR reaction was performed using Real-time PCR (Stratagene MX3005P) in the total volume of 25 µl as follows; 12.5 µl 2x QuantiTect SYBR Green PCR Master Mix (Qiagen, Germany), 0.25 µl Reverse transcriptase (Thermo Fisher), 0.5 µl for each Forward and reverse primer (20 pmol), 8.25 µl RNase Free Water and 3 µl Template RNA. The qRT-PCR product was analyzed and Amplification curves and ct values were determined by the stratagene MX3005P software. To estimate the variation of gene expression on the RNA of the different samples, the CT of each sample was compared with that of the control group according to the "ΔΔCt" method stated by Yuan et al **[33]** using the following ratio: (2-DDct). Whereas ΔΔCt = ΔCtreference – Δcttarget, ΔCt target = Ct control – Ct treatment and ΔCt reference = Ct control- Ct treatment, E: efficiency of amplification.

Water sampling and analysis

Water samples were collected from three localities, the first being Qarun Lake (n=12), the second being Wadi El Rayan (n=3), and the third being the fish farms (n=3). Samples were transported to the laboratory of the Veterinary Hygiene and Management Department, Faculty of Veterinary Medicine, Cairo University. Chemical water quality parameters for water samples were determined according to Standard Methods described by APHA [34]. pH was measured using a pH meter (Jenway® Model: 550). Total hardness was measured using the EDTA titration method, Chlorides (CL) were estimated by “Argentometric method”, Ammonium, nitrite, and nitrate were analyzed using specific kits (HANNA® instruments) Sulphates (SO_4) concentration was determined by “The gravimetric methods with the drying of residues” and Phosphate was estimated by using “stannous chloride” according to SMWW [35]. Water samples were taken with a water sampler from different localities in each of the studied sites between 10.00 and 12.00 am at a depth of 30 cm below the water surface and stored at 4ºC in a clean 1000 ml sampling glass bottles according to [36, 37].

Histopathological Examination

Tissue Specimens from the gills of parasitological infected fish were fixed in 10% neutral buffered formalin for 24 h. The fixed tissues were subjected to trimming, processing and sectioning at 5 um thickness. Tissue sections were stained with H&E [38,39], and examined with a light microscope. Microphotographs were captured with an Olympus camera fixed to the microscope.

Results

Macroscopical and clinical examination of fishes

The collected Tilapia *zillii* were apparently healthy and had no external lesions except some of them had dark skin coloration, petechial hemorrhages on the isthmus and belly and excessive mucus secretion (Fig. 2). When we open the gill cover the gills have swelling and redness in color and deformed. However, the examined *S. aegyptiaca* fish showed *Livoneca redmanii* macroscopically attached to gill filaments (n=1-2) as illustrated in Fig. 3

Parasitological identification

Trichodina spp., *Cichlidogyrus tilapiae*., *Gyrodactylus* spp. and *C. formosanus* were observed in wet-mount preparation of *T.zillii* gills. *Trichodina* spp. identification was based on the body shape which was of a medium-sized and of a disc- to bell-shaped observed between the gill lamellae (Fig. 4A). The body of *Gyrodactylus* sp. was elongated, at the anterior end, three pairs of cephalic glands at two posterior ocelli (eyespots) with two small inconstant anterior ocelli. Male copulatory complex with penis and accessory piece. Median posterior testis with Vas deferens on the right side, not encircling the intestinal branch. Median pre-testicular ovary and ventral sub-median vaginal opening. At the posterior end, there are two pairs of anchors, one dorsal and one ventral with two transverse bars, dorsal with two auricles while the ventral one is V-shaped with fourteen marginal hooks (Fig. 4B). The observed *Cichlidogyrus tilapiae* is characterized by an elongated body at the middle double-pointed anterior end and no eyespots. A large opisthaptor consists of two centrally positioned approximately parallel large hooks joined by two connecting bars, a simple dorsal bar and an approximately triangular-shaped ventral bar. There are 16 marginal hooks positioned around the periphery of the opisthaptor. **(**Fig. 4C). *Centrocestus formosanus* EMC identification based on an X-shaped excretory bladder and 32 circumoral spines (Fig. 4D). *Livoneca redmanii* was morphological of body length ranging from 10-15mm, having pale yellowish creamy grayish color with brown colored chromatophores forming thin stripes along posterior margins of the cephalon, pereon, and pleon segments, excluding pleotelson. Pleonites decreasing in width towards the posterior also have large eyes, well-developed, oblong and set wide apart (Fig. 3B).

Prevalence of parasitic infection

Prevalence of total parasitic infection among examined fishes

The total number of examined fish was 274 fish (205 *T. zillii* and 69 *S. aegyptiaca* fish), and the number of infected fish was 93 with a percentage of infection of 33.9 %. On the level of fish species, the percentage of infection was 44% in Tilapia *zillii*, and 4.3 % in *S. aegyptiaca*.

Prevalence of isolated ectoparasites among examined fish

Trichodina spp. were detected in 9 fish with a 4.3% percentage of infection; *Gyrodactylus* spp. and *Cichlidogyrus tilapiae* were detected in 23 and 28 infected fish with a percentage of infection of 11.2 % and 13.6 % respectively; *C. formosanus* EMC was observed in 30 infected fish with a percentage of infection 14.6%. *Livoneca redmanii* was observed in 3 infected *S. aegyptiaca* fish only with a percentage of infection of 4.3% (Fig. 5) .

Relation between infected fishes with different parasites in different collection localities

The total number of examined fishes from Lake Qarun and Wadi El Rayan were 117 and 157 fishes respectively where the total prevalence of parasitic infection was 41.8 % and 28 % (Fig.6). Regarding isolated parasites, *Livoneca redmanii* was detected only in *S. aegyptiaca* fish collected from Shakshouk area in lake Qarun with 4.3% percentage of infection. *Trichodina* spp., *Gyrodactylus* spp., *Cichlidogyrus tilapiae*; and *Centrocestus formosanus* encysted metacercariae were isolated from *T. zillii* collected from private farms of Lake Qarun with prevalence % of 18.75%, 8.3 %, 58 % and 10.41 % respectively. However, *T.zillii* collected from Wadi El Rayan was infected with *Gyrodactylus* spp. 12% and *C. formosanus* 15.9% as illustrated in Fig. 7.

Immune Gene expression analysis

TNF-α and IL-1β gene expressions of infected gills with different parasitic groups were determined in Fig. 8. *TNF-α* gene was upregulated 9.71-fold, 6.94-fold- fold and 8.15-fold in the case of *Livoneca redmanii*, C. *formosanus* EMC, and *Trichodina* spp. infection respectively when compared with the control group (p value=0.000). *IL-1β* levels were upregulated 7.09- fold, 5.17- fold and 6.04- fold in the case of *Livoneca redmanii*, C. *formosanus* EMC, and *Trichodina* spp. infection respectively when compared with the control group (p value=0.000) (Table 1).

Water quality analysis

The physicochemical study of water samples from four locations at Lake Qarun (Table 2) indicated that the pH ranged from 7.4 at site 3 to 8.14 at site 4. The salinity ranged from 26 ppt at site 2 to 29 ppt at site 4. The greatest TDS in the range of 20.03±1.16 g/l was found at Site 4. The findings of the present investigation demonstrated that the average amounts of ammonia, nitrite, nitrate, and phosphate exceeded permitted limits at all sites in Lake Qarun. The concentration levels of COD ranged from 4.25 to 20.11 mg/l. The maximum COD value in Lake Qarun was 20.11 mg/l at site 2. Table 3 presents comparative physical and chemical water analysis results obtained over the study period from several places (Lake Qarun, Wadi El Rayan, and Lake Qarun fish farms). The pH readings didn't significantly differ between the various locations. Ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), chloride, and phosphate concentrations above the specified allowed limits. Chloride values in the farm water and the other source waters were not significantly different. On the other hand, there were significant differences ($p<0.05$) in TDS, salinity, ammonia and nitrate levels among the study sites.

Histopathology

Histopathological results revealed severe and diffuse hyperplasia of gill lamellae associated with the presence of *Trichodina* spp., some lamellae show a degree of degeneration, and the branchial blood vessels are widely dilated and congested as shown in Figure. 9 (A-D). Encysted C. *formosanus* was observed in the gill filaments which cause massive and diffuse necrosis and hyperplasia to the gill lamellae and widening and congestion of gill blood vessels, the large number of the encysted parasites in the gill arch cause the presence of inflammatory cell reaction (Fig. 9E-L) .

Discussion

The Egyptian fresh and mariculture industry is exposed to serious threats due to infection with parasitic diseases that reduce the quality and quantity of fish production, causing drastic economic losses as well as presenting a prominent health hazard among fish consumers [41,42].

The comprehensive examination of clinical findings in the present study unveiled a range of clinical manifestations and parasitic investigation in *Tilapia zillii*, and *S. aegyptiaca* collected from different localities (Lake Qarun, and Wadi El Rayan) in Fayoum governorate during the studying period from October 2020 to June 2022. Tilapia culture is one of the most important fast-growing aquacultures worldwide. *O.niloticus*, *T. zillii* and *S. aegyptiaca* are the most common species found in Lake Qarun and Wadi El Rayan. [43]. Throughout the 20th century, fish disappeared gradually from all sites of Lake Qarun due to a heavy abundance of crustacean parasites that caused damage to fish stock in the lake from all prospectives [6], it not only caused mortalities to the small fish fries but also affect the growth and reproduction of inhabitant fish species. Parasitic diseases can cause respiratory distress; especially when the infection is found in gills; weight losses, malformations, and secondary bacterial and fungal infections, thus hindering fish marketability and causing serious economic losses [41].

In our study, the clinical manifestations observed on fish skin are in the form of skin discoloration, hemorrhages and excess mucous secretion in accordance with that mentioned by Fisheries et al [44] in ectoparasitic infected fishes.

The total prevalence of parasitic infection was 33.9 % in *T. zillii* and *S. aegyptiaca*, collected from the Qarun and Wadi El Rayan lakes, respectively. These results are lower than that mentioned by Sayed et al [45] and Abdelrahman et al [46] where the overall infestation rate with variable parasites was 68.5% and 82% respectively in *Oreochromis niloticus* from Kafr El sheikh governate and Assiut governorate in Egypt, 54% and 78% in *Synodontis serratus* and *Synodontis schall* catfish from Lake Nasser in Aswan governorate [47]. The difference in prevalence percentage may be attributed to fish species variations, seasonal variations, differences in the periods of investigation, time and site of fish sampling, geographical distribution of fish hosts and parasites and environmental changes [5].

Wet mount and light microscopical examination were conducted for preliminary parasitological identification. *Trichodina* spp., are commensals ciliated ectoparasites. However, heavy skin or gills infestation can cause tissue damage. *Trichodina* was observed in the gills of collected *Tilapia zillii* with a prevalence of infection of 4.3%, which is nearly similar to the results of Khallaf et al. [48] where

Trichodina percentage of isolation was 4.82%; 4.36% and 5.76% from Tilapia fish collected from different localities in Beheira governorate, and Abdelrahman et al. [46] that recorded external protozoa infection rates for *Trichodina* were 6%. However, a high prevalence of *Trichodina* infection (45%and 83.33%) was recorded in Fahaka pufferfish and Nile tilapia in Egypt as reported by Abdel-Baki et al. [13] and Younis et al. [49].

Monogenean is a class of Platyhelminthes parasitic trematodes, that have a direct life cycle where fish is the final host [48]. It is mostly found attached to gills and external surfaces of fresh and marine water fishes via opisthaptor causing pathological lesions and tissue damage. [50,51]. In our study, infestation with monogenetic trematodes *Gyrodactylus* spp., and *C. tilapiae* have been found in wet mount preparations of gills of *Tilapia zillii* at a percentage of 11.2 % and 13.6 % respectively, higher than that mentioned by Khallaf et al. [48], where the monogenean trematodes *Gyrodactylus* spp. were isolated from Tilapia species with the prevalence of infection(1.49%,1.44% and 1.65%) respectively from different areas along Nile River in Beheira governorate; Al Bassel [52] who reported monogeneans percentage (1.77%) in Fayoum governorate Egypt and Abdelrahman [46] who mentioned that prevalence percentage was 5% Gyrodactylus and in Nile tilapia. In contrast, Adawy et al. [53] recorded a higher prevalence (26%) of monogenean parasites in different marine fish species.

*Centrocestus formosanu*s is a digenetic trematode with a complex life cycle, involving invertebrate and vertebrate hosts, and even humans. In particular, it causes gill lesions in different freshwater fish species [45,18], moreover, it has public health significance causing gastrointestinal symptoms in infected humans fed on raw or unproperly cooked fish meat, which describes the potential risk for human health [17]. In our study, the prevalence of infection with *C. formosanus* EMC was 14.6%. The higher percentage of infection with *C. formosanus* than other isolated parasites recorded in our study may be attributed to the presence of piscivorous migratory birds in El Fayoum aquatic environments that act as definitive hosts at which the adult trematode developed as mentioned by Pace et al. [17]. Our results were nearly identical to that of Hamouda [47] where *C. formosanus* EMC prevalence of infection was 23% and 11%, but lower than that of Younis et al. [49], Abou-okada et al. [54] and Shafiq et al. [55] where the EMC of *C. formosanus* was detected in Nile tilapia fish gills and muscles with isolation percentage of 72.2%, 88% and 35%, respectively.

Crustacean parasites are one of the great challenges facing the fish culture industry in Lake Qarun. Cymothoid isopods are obligate parasites. Heavy infestations reduce fish growth index and body weight. Our Results revealed a low prevalence of 4.3% with *Livoneca redmanii* infestation among *S.aegypteacae* fishes in Qarun Lake, this agreed with the results of Ali et al. [56] that most of the *S. aegyptiaca* fish collected from Lake Qarun was infested with Isopods, also come in accordance with Abdel-Latif et al. [57] that rate of Isopods infestation in *T. zillii* collected from Qarun lake was 6%. But unlike the results of Mahmoud et al. [23] the Cymothoid isopods total infestation rate was 19% in *Tilapia zillii*, *Solea* spp., *Mugil Capito* and *Sardinella* spp. collected from the Mediterranean Sea; and results of Shaheen et al [58], Helal and Osama [6] and, Khalaf-Allah et al [25] where 41.1%; 47% and 20.33% of collected fish from different areas including lake Qarun were infected with isopods. The difference in isopods prevalence percentage is directly related to the time of our current investigation, during which there was a reported decrease in the number of inhabitant fish species as a result of the extensive Lake pollution.

Molecular tools using quantitative real-time polymerase chain reaction (qRT-PCR) have a great concern in the detection of cell-mediated immunity in infected fish by measuring the levels of TNFα and IL-1β pro-inflammatory cytokines [20]. The increase in levels of these genes in infected fish tissue acts as an indicator of activation in immunological cells such as macrophages and neutrophils in response to parasitism [59, 60]. In our study, a directly proportional relationship between parasitized fishes with isopoda, *Trichodina* spp. and *C.formosanus* and enhanced immune response was estimated. Our results revealed the upregulation of TNFα and IL-1β gene expression as a stress-related response to parasitic infection in *T. zillii* and *S. aegyptiaca* in agreement with Younis and Mahmoud, et al [49, 61] in case of different parasitic infections.

Water quality specifications in tilapia culture are an essential consideration in determining the health and production of fish, as they are significantly impacted by management practices [62, 63]. These practices can have a negative impact on water temperature, dissolved oxygen, ammonia, nitrites, nitrates, salinity, pH, and alkalinity. These changes can create stressful conditions that compromise the host's immunity and ultimately result in death [64, 9]. Several reports have been made regarding the precarious quality of water in relation to tilapia bacterial, viral and parasitic diseases [65, 66, 64, 67, 1]. Ectoparasites, including *Trichodina* spp. and monogeneans, exhibited the highest statistical correlation with variations in water quality indices [68].

Ammonia is the second most critical gas in fish culture, behind dissolved oxygen; its impact on aquaculture production is substantial. The water samples examined in this study from several locations in Lake Qarun revealed the existence of detrimental water quality indices, including elevated concentrations of ammonia, nitrite, nitrate, and phosphate. The ammonia concentrations observed in this investigation varied from 2.19 to 3.73 mg/L. The allowable concentration of ammonia is 0-0.05 mg/L, with an acceptable threshold of less than 0.5 mg/L [23]. The nitrite levels observed in this investigation exhibited variability, with the greatest concentration recorded at 0.44 mg/L, beyond the allowed limit. The average nitrate levels at sites 1, 2, and 3 (3.46–12.59 mg/L) exceed the permissible limit. Table 3 indicates that the chemical analysis of the sampled water reveals elevated concentrations of ammonia, nitrite, and nitrate, with a significant difference ($P< 0.05$) across the several sampling locations (Lake Qarun, Wadi El Rayan, and Lake Qarun Fish Farm) in terms of TDS, salinity, ammonia, and nitrate levels. The significant alterations in water Salinity significantly impact the biota of Lake Qarun [25]. The continuing discharge of sewage and agricultural runoff into Lake Qarun, coupled with the subtropical climate of the Fayoum governorate, results in persistent water evaporation. Over time, this leads to an accumulation of organic compounds, inorganic salts, heavy metals, and other pollutants, which may contribute to the reduction of dissolved oxygen levels in the water. All the aforementioned variables culminated in a reduction of the fish population in the lake [6, 69]. Significant reduction of dissolved oxygen may also result from the decomposition of organic matter that enters aquatic systems [70, 71]. In the study by Benli et al [72], elevating ammonia concentrations to 1, 2, 5, and 10 mg/L resulted in histological alterations, primary hyperplasia of the gill epithelium and degenerative abnormalities in the liver and kidneys. Elevated NH_3 concentrations (1.85–3.38 mg/L) are associated with outbreaks of *Trichodina* sp., *Cichlidogyrus* spp., *Gyrodactylus cichlidarum, Vorticella* sp., and *Enterogyrus malmbergi* [73,74,75] associated elevated water quality parameters, including nitrite (0.04 mg/L), un-ionized ammonia (0.8 mg/L), hydrogen sulfide levels (153.1 mg/L), and organic matter content (3.79 mg/L), with outbreaks of co-infection involving the ectoparasite *Gyrodactylus cichlidarum* and the bacterium *A. hydrophila*. Nitrite accumulates in aquaculture systems and can be detrimental to aquatic organisms [76,77]. In Nile tilapia, increased nitrite levels of 5.0 mg/L at 24 and 96 hours prompted the secretion of cortisol hormones. The elevation of cortisol has been associated with compromised immunological function and, as a result, diminished disease resistance [78]

Poor water quality or pollution-induced physicochemical variations can create favorable conditions for pathogen survival or impair the immunological response of fish [79,9]. The lack of proper management practices significantly elevates the likelihood of pathogen transmission and proliferation in tilapia aquaculture**.**

The result of the histopathological investigation shows variable degrees of pathogenicity according to the presence of the parasite in the host’s body. The main observed pathological changes were in gills in the form of necrosis and hyperplasia to the gill lamellae, widening and congestion of gill blood vessels, and lamellar degeneration, similar to that observed by Mahmoud et al. [80] and Shaheen et al [58] in case of isopods and Trichodina spp. Infection. Muscular diffuse vacuolations and degenerations with the presence of encysted parasites between muscle bundles were seen in fish infected with *C.formosanus* in agreement with Purivirojkul and Shafiq et al. [8**1**,55].

Conclusion

To sum up, this study’s results offer a thorough summary of the most common parasitic spp. present in *T.zillii* and *S. aegyptiaca* fish collected from Lake Qarun and Wadi El Rayan lakes under bad water quality criteria. Parasitological identification revealed the isolation of different Ectoparasites, and the histopathological picture clarifies and confirms the damaged effects of these parasites on fish gills. The immunological response of fish to parasitism was measured via the detection of the increase in *TNFα* and *IL-1β* genes levels. These all highlight how important it is to comprehend how these parasites can affect fish health, productivity and the aquaculture industry. However, the presence of these parasites in fish-rearing water requires financial and management efforts by controlling agricultural and sewage runoff and restocking of lake with healthy fish fries.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Cairo University, Egypt.

TABLE1. TNF-α and IL-1β Immune genes expression (mean ±SE)

Parasite	TNF-α	IL-1β
Livoneca redmanii	10.25 ±0.011[b]	7.63 ±0.023[ab]
C. *formosanus* EMC	7.48 ±0.011[d]	5.71 ±0.991[b]
***Trichodina* spp.**	8.69 ±0.009[c]	6.58 ±0.010[b]
Control	0.54 ±0.114[e]	0.54 ±0.114[c]
P* value	0.000	0.000

*Means followed by different lowercase letters in the rows are statistically different ($p \leq 0.05$).SE; standard error

TABLE 2. Mean values ±SE of physical and chemical parameters of Lake Qarun water from four different sites during the study period.

Parameters	Site 1	Site 2	Site 3	Site 4	Permissible limits according to EPA [40]	**P* value
pH	7.93±0.26	8.03±0.37	7.4±0.32	8.14±0.22	6.5 – 8.5	0.37
TDS (g/l)	17.6±0.59	15.96±0.57	17.26±1.39	20.03±1.16	--	0.10
Salinity (ppt)	27.2± 1.2	26± 1.5	27.5± 1.1	29± 0.9	--	0.134
Chloride (mg/L)	983.33±346.8	1272.33±442.98	506.67±93.87	401±6.66	250 mg/l	0.18
Ammonia (mg/L)	3.16±0.72	2.56±0.69	3.73±0.82	2.19±0.40	0.05–0.5	0.44
Nitrite (mg/L)	0.44±0.27	0.12±0.017	0.12±0.06	0.12±0.049	0.01–0.03	0.36
Nitrate (mg/L)	10.05±1.80	8.05±1.59	12.59±0.43	3.46±3.07	2–5 mg/l	*0.056
Sulphate (mg/L)	193.3±43.33	330±49.3	200±20.82	218±11.29	200	0.075
Phosphate (mg/L)	3.3±0.67	3.96±0.94	1.67±0.32	1.98±0.38	0.5–0.7	0.1
COD (mg/L)	13.4±1.06	20.11±2.70	18.3±0.88	4.29±0.35	7	*0.00035

SE; standard error COD: chemical oxygen demand TDS: total dissolved solids. *Means followed by different lowercase letters in the rows are statistically different ($p \leq 0.05$).

TABLE 3. Comparative physical and chemical water analysis from different localities (lake Qarun, Wadi El Rayan, and lake Qarun fish farms) during the study period.

	lake Qarun			**Wadi El Rayan**			**Fish farms**			
	Min.	**Max.**	**Mean ±SE**	**Min.**	**Max.**	**Mean ±SE**	**Min.**	**Max.**	**Mean ±SE**	***P* value**
pH	6.9	8.7	7.87±0.15	7.5	8.3	7.88±0.10	7.4	7.6	7.5±0.06	0.157
TDS (g/l)	15	21.9	17.725±0.61[a]	1.2	2	1.56±0.14[c]	4.90	7.55	6.4±0.78[b]	*0.000
Salinity (ppt)	26	29	27.33± 1.3[a]	3	3.7	3.22± 0.65[c]	6.5	8.8	7.76± 1.43[b]	*0.00
Chloride	380	1900	790.83±161.83	600	900	675.83±46.23	450	500	483.3±16.67	0.136
Ammonia (mg/L)	1.21	5.3	2.91±0.33[b]	0.22	1.4	0.67±0.18[c]	2.9	4.3	3.63±0.41[a]	*0.001
Nitrite (mg/L)	0	0.98	0.20±0.07	0.2	0.4	0.29±0.027	0.2	0.34	0.25±0.04	0.787
Nitrate (mg/L)	0	13.4	8.54±1.31 [a]	0.4	1.1	0.63±0.11[b]	8	15	11±2.08[a]	*0.003
Sulphate (mg/L)	120	420	235.416±22.36	120	190	148.33±12.22	130	190	160±17.32	0.072
Phosphate	1.23	5.23	2.73±0.39	0.82	2.2	1.47±0.21	2.09	2.97	2.39±0.29	0.087
COD (mg/L)	3.89	25	14.03±1.96	11.4	16	14.16±0.71	15.79	17.23	16.34±0.44	0.16

Min. minimum; Max. maximum; SE: standard error; COD: chemical oxygen demand; TDS: total dissolved solids. *Means followed by different lowercase letters in the rows are statistically different ($p \leq 0.05$).

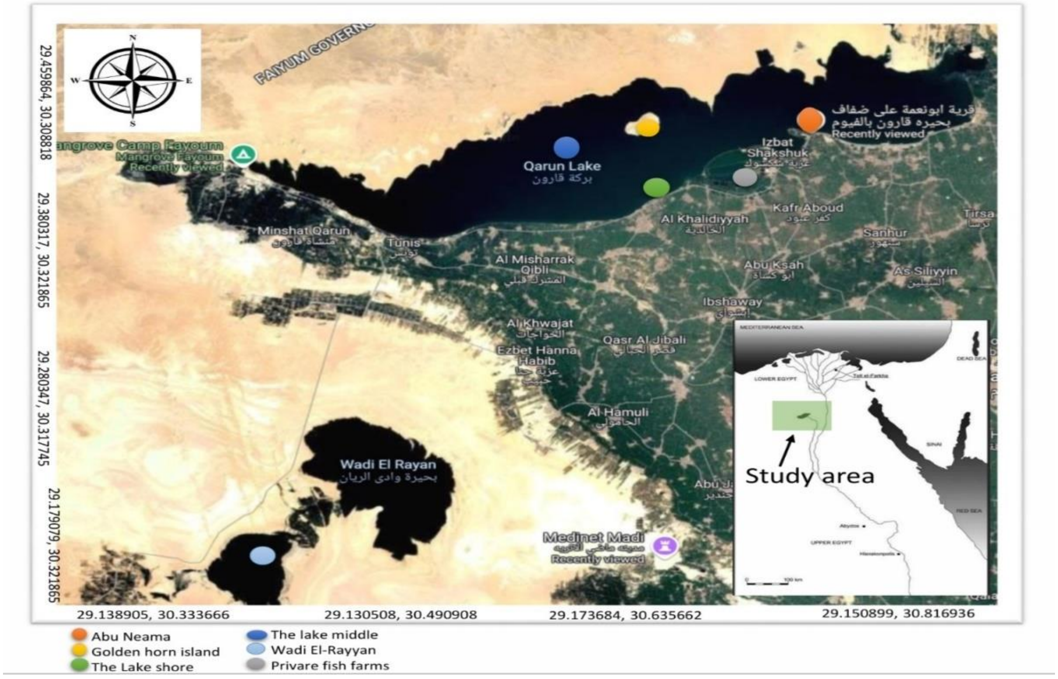

Fig. 1. The geographical distribution of sampling sites (Map was generated using Google Earth Pro, version 7.3.3.7692, https://www.google.com/earth/versions/."

Fig.2. A: *T. zillii* showing petechial haemorrhages; B) *S. aegyptiaca* fish showing skin darkness

Fig.3. A) *Livoneca redmanii* (Isopoda, Cymothoidae) on *S. aegyptiaca* attached to gills; B) Isolated *L. redmanii* from investigated *S.aegypteacae* (Dorsal view).

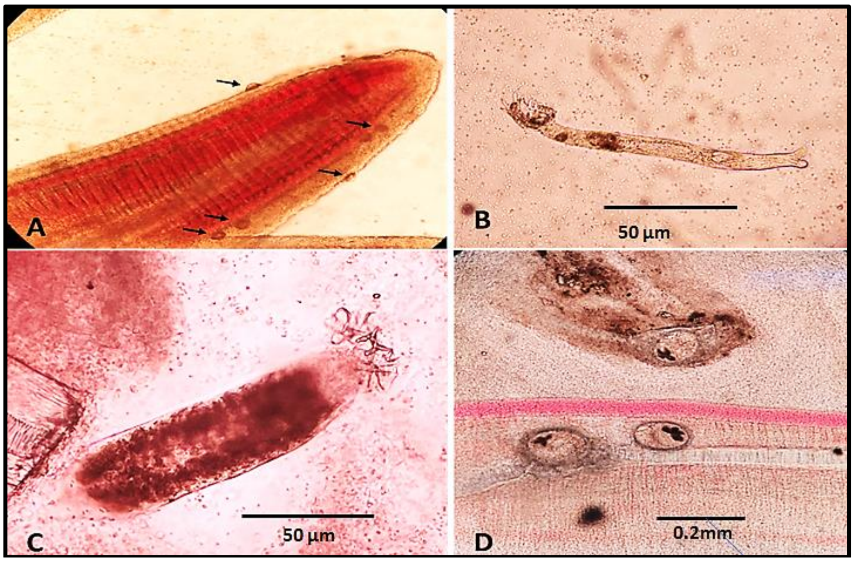

Fig 4. Wet mount preparation of gills of *Tilapia zillii* showing A) *Trichodina* spp. scattered along gill lamellae (arrows); B) *Gyrodactylus* sp.; C) *Cichlidogyrus tilapiae*; D) *Centrocestus formosanus* EMC scattered along gill lamellae of investigated fishes. Scale bare B-C) 50 micron. D) 0.2mm.

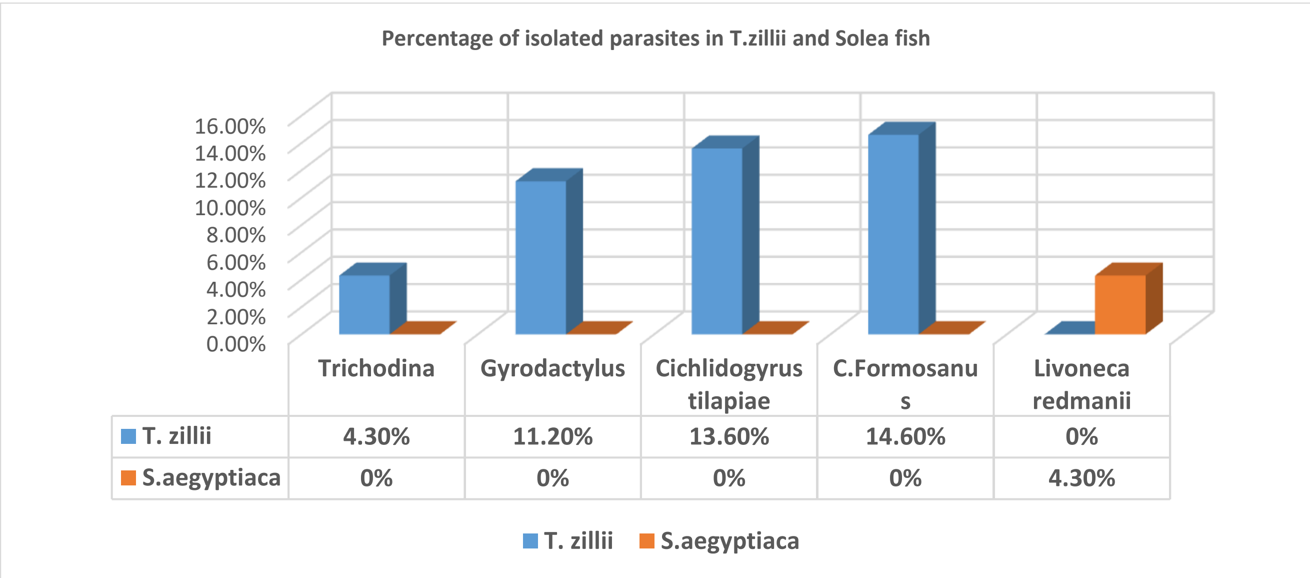

Fig.5. showing the Percentage of parasitic infection in Tilapia *zillii* and *S. aegyptiaca* in relation to number of examined fish species

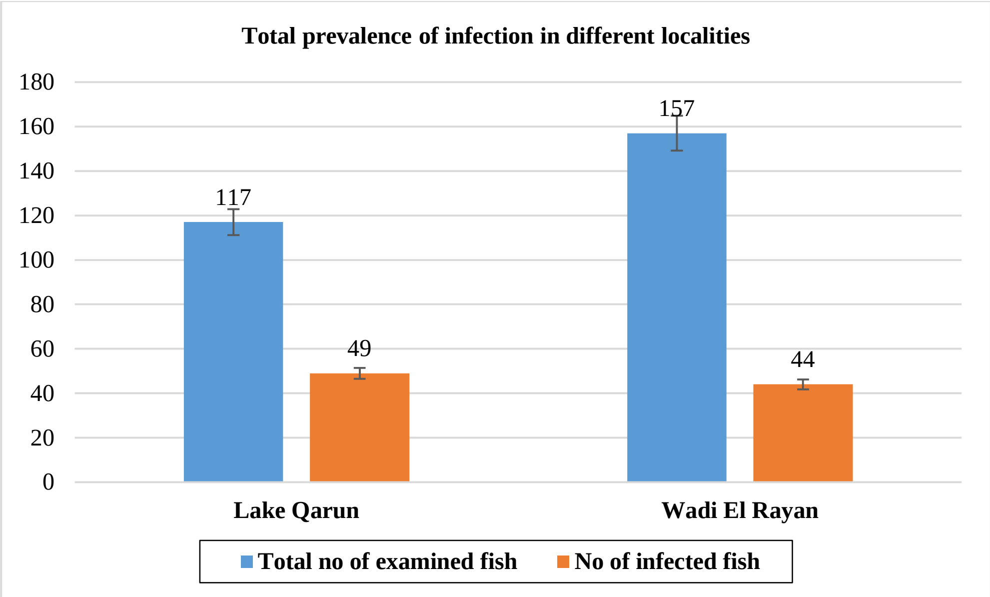

Fig. 6. Showing the total prevalence of infection in lake Qarun and Wadi El Rayan

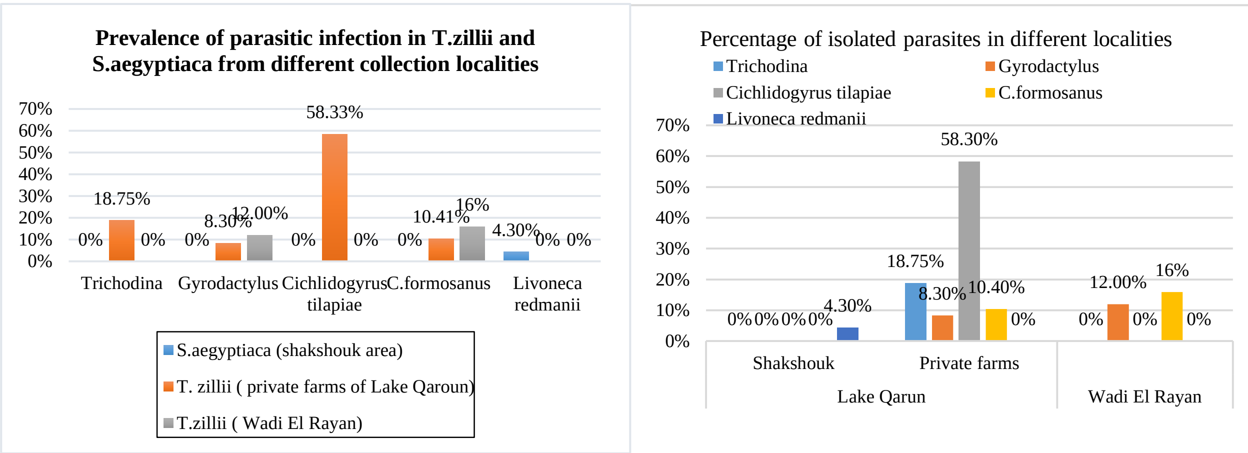

Fig.7. Showing the prevalence of parasitic infection in relation to collected fish species from different localities

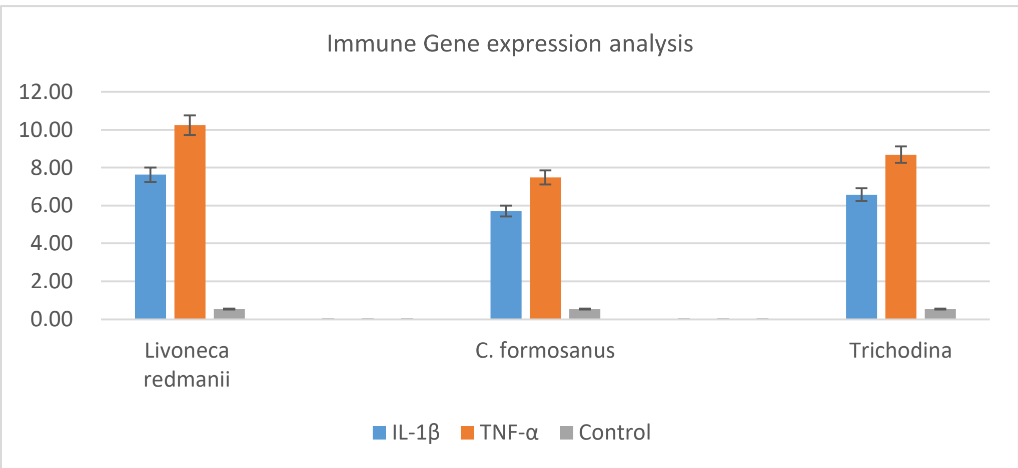

Fig.8. Tumor necrosis factor-α (TNF-α) and Interleukin-1β(IL-1β) genes expression in fish gills infected with *Livoneca redmanii, C. formosanus* EMC and *Trichodina* spp.

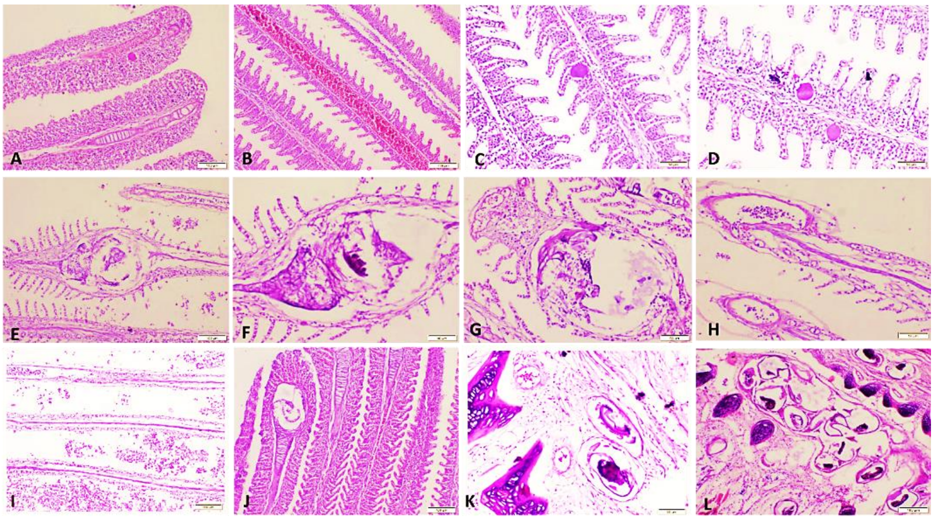

Fig. 9. (A) Severe and diffuse hyperplasia of gill lamellae associated with presence of *Trichodina* spp., some lamellae show degree of degeneration (scale bar = 100 um); (B) Gill blood vessels are widely dilated and congested, note the moderate hyperplasia of gill lamellae (scale bar = 100 um); (C) A higher magnification shows moderate hyperplasia of gill lamellae and presence of the parasite (scale bar = 50 um); (D) Note the presence of parasites and the associated hyperplasia of gill lamellae (scale bar = 50 um); (E) Presence of large, encysted parasite in the gill filament associated with damage and necrosis of the cells (scale bar = 100 um);(F, G) A higher magnification shows the encysted parasite (scale bar = 50 um);(H) Severe widening of gill blood vessel and necrosis of gill lamellae (scale bar = 50 um); (I) Massive and diffuse necrosis of gill lamellae and congestion of blood vessels (scale bar = 100 um); (J) Presence of encysted parasite in the gill and hyperplasia of the gill lamellae (scale bar = 100 um); (K) Presence of two encysted parasites in the gill arch (scale bar = 50 um); (L) Large number of encysted parasites in the gill arch with presence of inflammatory cell reaction (scale bar = 100 um).

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تحليل مقطعي ودراسة مناعية للإصابات الطفيلية في أسماك موسي وأسماك البلطي الزيلي من بحيرتي قارون ووادي الريان

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الملخص

تتعرض بحيرات قارون ووادي الريان في الوقت الحاضر لتغيرات بيئية ضارة أدت إلى فقدان مخزون الأسماك. في هذه الدراسة، سجلنا الإصابات الطفيلية الأكثر شيوعًا في هذه البحيرات فيما يتعلق بمعايير جودة المياه السيئة والآثار الطفيلية والمرضية المرتبطة بها. تم فحص إجمالي عدد 274 من الأسماك: 205 من أسماك البلطي الزيلي و 69 من أسماك موسي تم جمعها عشوائيًا من بحيرات وادي الريان ومزارع الأسماك الخاصة ومنطقة شكشوك في بحيرة قارون بمحافظة الفيوم، مصر. بلغ معدل انتشار العدوى الطفيلية 33.9٪. كانت جميع الأسماك التي تم فحصها إيجابية لواحد أو اثنين من الإصابات الطفيلية المختلطة. Trichodina spp. و Gyrodactylus spp. و تم الكشف عن Cichlidogyrus tilapiae و Centrocestus formosanus encysted metacercaria و Livoneca redmanii في 4.3٪ و 11.2٪ و 13.6٪ و 14.6٪ و 4.3٪ من الأسماك المصابة على التوالي. تم الكشف عن الاستجابة المناعية للأسماك للتطفل من خلال زيادة مستويات جينات TNFα و IL-1β مع اختلاف كبير (P = 0.000). تشير نتائج جوده المياه الفيزيائية والكيميائية التي تم الحصول عليها من الأماكن المدروسة إلى أن تركيزات الأمونيا والنتريت والنترات والفوسفات كانت أعلى من الحدود المسموح بها المحددة. وصفت الآفات النسيجية المرضية المرتبطة بالإصابة الطفيلية تضخمًا شديدًا وتنكسًا ونخرًا في صفائح الخياشيم؛ احتقان الأوعية الدموية الخيشومية المرتبط بوجود Trichodina spp. و Encysted C. formosanus في الخياشيم. كل هذا يسلط الضوء على مدى أهمية فهم كيفية تأثير هذه الطفيليات على صحة الأسماك وإنتاجيتها وصناعة الأحياء المائية. ومع ذلك، فإن وجود هذه الطفيليات في مياه تربية الأسماك يتطلب المزيد من الجهود المالية والإدارية

الكلمات الدالة: التغيرات البيئية، TNFα، التحليل الفيزيائي والكيميائي، الهيستوباثولوجي، الفيوم.