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# Studies on Some Parasitic Diseases in *Oreochromis niloticus* Fish Hatchery with Emphasis to Life Stages

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ABSTRACT

This study was conducted on 210 *Oreochromis niloticus* (*O. niloticus*) of different life stages including (100 fry, 100 fingerlings and 10 broodstocks) obtained from a private fish hatchery at Kafer El-Sheikh Governorate, Egypt; during August 2014. The hatchery complains 30% mortality among fry and fingerlings while no mortalities was recorded among broodstocks. Parasitological examination revealed heavy infestation with *Triochodina* species (sp.) in all examined life stages at a prevalence rate 100%. In addition, *Gyrodactylus* sp. was recorded in gills of fry, fingerlings and broodstocks at a rate of 5, 12, and 10 %, respectively. Kidneys and gills of all examined life stages showed heavy infestations with *Myxosporean sp*., with 100 % prevalence rate. *Haemogregarina sp.* was described in the blood of fingerlings and gill tissues of broodstocks. Additionally, Encysted metacerceria was observed in gills of broodstocks. The recovered parasites were demonstrated hisopathologically in the gill and kidney tissues of the examined fish. The histopathological examination revealed that the infested gills exhibited serious lesions such as hyperplasia and hypertrophy of the lining epithelial cells of the gill filaments, fusion and necrosis of secondary lamellae and vasodilatation. The lining epithelium of the renal tubules showed degenerative and necrotic changes with the presence of various developmental stages of myxosporidia. In conclusion, fry and fingerlings exhibited high mortalities, while no mortality was recorded among broodstocks, regardless the intensity of infestation and severity of pathological alterations which was intense in broodstocks.
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## Introduction

Parasitic diseases constitute the largest sector of fish diseases in warm water fishes in Egypt, reaching 80 % (Eissa, 2002). Although the majority of fish ectoprotozoa is commensally, but they induce serious diseases and mass mortalities especially in fry and fingerlings under stress condition (Khan, 2004; Eissa *et al*., 2013). Generally, fish parasites result in economic losses not only due to mortalities, tissue damage, and growth reduction but also from treatment expenses, which limited the expansion of aquaculture.

Trichodinosis is one of the ectoparasitic protozoal diseases caused by *Trichodina sp*., they have a direct life cycle and they reproduce by binary fission. Interestingly, most trichodinids are not pathogens, but under certain environmental conditions or when the fish are stressed by other factors, the parasite increases greatly its rate of infestation among fish and can become pathogenic and result-

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ing in hyperplasia and necrosis of gill epithelium and cause mass mortality of infested fish (Abdel-Meguid, 2001). This parasite was extensively isolated from gills of both freshwater and marine fishes (Xu *et al*., 2002; Yemmen *et al*., 2011; Soliman *et al*., 2013).

Gyrodactylids are viviparous monogenean ectoparasites, parasitized skin and gills resulting in mortalities especially among young fishes (Eissa, 2002; Bruno *et al*., 2006). Myxozoans are considered one of the most economically important groups of microscopic metazoan parasites which parasitize invertebrates and fishes either freshwater or marine-water resulted in death of the host (Bassey, 2011). Fry stage is often the most susceptible stage to myxosporidiosis (Eissa, 2002). Different *Myxosporean sp.* have been recorded in Egypt from different organs of wild and cultured *O. niloticus* (Soror, 2008; Shehab El- Din 2008; Matter *et al*., 2013). Severe infestation with *Myxosporean sp.* led to renal degenerative changes (Soror, 2008), gill hypertrophy and fusion of gill filaments resulting in respiratory disorders (Rukyani, 1990).

*Haemogregarina* blood parasite is apicomplexan protozoa, broadly distributed among vertebrate hosts, including fishes (Davies and Johnston, 2000). It mainly infects marine fishes but some species as *Haemogregarina majeedin sp.* was recorded in freshwater fish (Al-Salim, 1993). Heavy infestation with *Haemogregarina* usually resulted in anemia and cachexia of the infested fish (Pazooki *et al*., 2007).

Fish act as both intermediate and final host for larval stages of many digenetic trematodes such as *Clinostomum tilapiae*, *Euclinostomum heterostomum* and *Posthodiplostomum cuticola* (Taher, 2009; Eissa, 2011). Encysted metacercariae causes severe gill damage, decrease respiratory efficiency and subsequently mortality in pond raised fish (Paperna, 1991).

The aim of this study was to investigate some external and internal parasites in *O. niloticus* fry and fingerlings that might be implicated in mortalities occurred in the hatchery. Furthermore, evaluation of histopathological changes caused by these detected parasites.

## Materials and methods

*Oreochromis niloticus* fry, fingerlings and broodstocks of average weight 0.3, 3.0±1.0 and 350.0± 15.0g, respectively were collected from private fish hatchery at El Rayad, Kafr El-Sheikh, Egypt during August 2014 (Fig. 1). The hatchery suffered from 30% mortality rate among fry and fingerlings while there were no mortalities among broodstocks.


Fig. 1. Map for Kafr El-Sheikh Governorate showing the place of fish collection (arrow).

In the hatchery protocol, broodstocks were reared in the earthen pond and just before spawning; the female and male broodstocks were transferred to the concrete ponds in greenhouse and stocked at a ratio (3:1), respectively (Fig. 2). After hatching, the fry were collected every week and transferred to the concrete pond for nursing and start feeding on 17 α methyltestosterone treated diets for production all male tilapia.

Fig.2. The hatchery showing the earthen ponds for rearing broodstocks and concert ponds for nursing fry.

A total number of 210 alive *O. niloticus* including (100 fry, 100 fingerlings and 10 broodstocks) were transferred in double skinned polyethylene bag pumped with oxygen, while the broodstocks were transferred in large plastic container suffi-

ciently supplied with oxygen to the wetlab of Fish Diseases and Management department, Faculty of Veterinary Medicine, Benha University, Egypt. Fry and fingerlings were kept in well prepared aquarium supplied with sufficient amount of dechlorinated tap water with continuous aeration. The freshly dead fry and fingerlings were examined daily. The whole batch of fry and fingerlings showed 100 and 70 % mortality, respectively within 3 days. The remaining 30 % fingerlings and the apparently health *O. niloticus* broodstocks were sacrificed and subjected to parasitological and histopathological examinations.

*Parasitological examination*

Blood samples were collected in heparinized microhaematocrite tubes (Vitrex, Hertev, Denmark) from 30 alive fingerlings by cutting the tail. The dry blood smears were fixed with methanol, stained with freshly prepared Giemsa stain and finally microscopic examination was carried out.

Tissue smears from gill filaments and kidneys of fingerlings and broodstocks were prepared with a drop of saline and covered with a cover slip (wet mount preparation) and examined microscopically. While, fry were taken as whole and squashed between two slides with a drop of saline. The positive smears were air dried, fixed with absolute methyl alcohol and stained with Giemsa stain according to Lom and Dykova (1992).

*Histopatholgical examination*

Tissue sections from the gills and kidneys of infected *O. niloticus* broodstocks and whole fry were fixed in 10% buffered neutral formalin for histopathological examination. The dehydration, clearing and infiltration processes of samples were carried out in an automatic tissue processor using a series of graded alcohol, two changes of xylene and, finally, in three consecutive series of molten wax. The samples were then embedded in paraffin wax and sectioned at 5 µm thickness. Sections were stained with Meyer's hematoxylin and eosin (Gridley, 1960) or a modified Giemsa's stain, and mounted with canada balsam. The prepared sections were then examined under a light microscope.

## Results

In the present study; within 3 days the cumulative mortalities of fry and fingerlings kept in the Lab. reached 100 and 70 %, respectively. The survived fingerlings showed signs of asphyxia with rapid opercular movement, surfacing and gasping.

The examined *O. niloticus* broodstocks showed no characteristic external lesions but some affected fish showed pale gills. Internally, enlargement of the posterior kidneys was observed. Some examined fish showed macroscopic yellowish white nodules embedded in the kidney.

*Parasitological examination*

Parasitological examination of fry, fingerlings and broodstocks gills revealed heavy infestation with *Trichodina sp* (Plate 1A). The prevalence rate was (100 %) in all examined life stages (Table 1). The intensity was 27-35 parasites per microscopic field for both fry and fingerlings while it was 15-25 for broodstocks.

*Gyrodactylus* sp. was recorded in gills of fry, fingerlings and broodstocks at infestation rate 5, 12, 10%, respectively (Table 1). The parasite appears flat with two elliptical projections at its anterior end. The posterior end (haptor) has two pairs of anchors and a number of marginal hooklets (Plate 1B).

*Myxosporean* spores were isolated from kidneys and gills of fry, fingerlings and broodstocks. The prevalence rate was 100 % among all examined

Table 1. The prevalence of different parasites isolated from *O. niloticus* fry, fingerlings and broodstocks

| | *Trichodina sp.* | | *Gyrodactylus sp.* | | *Myxosporean sp.* | | *Haemogregarina sp.* | |
|---|---|---|---|---|---|---|---|---|
| Life stages | (N) | (%) | (N) | (%) | (N) | (%) | (N) | (%) |
| Fry | 100 | 100 | 100 | 5 | 100 | 100 | - | - |
| Fingerlings | 100 | 100 | 100 | 12 | 100 | 100 | 30 | 10 |
| broodstocks | 10 | 100 | 10 | 10 | 10 | 100 | - | - |

N= Number of Examined

%= Percent of infestation

stages (Table 1). The *Myxosporean* spores showed diverse morphology. The spores appeared ellipsoidal, ovoid or round contains two equal pyriform or oviform polar capsules (Plate 1C). Moreover, spores with three spherical polar capsules at the anterior end were also demonstrated in the renal tissues.

*Haemogregarina sp.* was recorded in blood of fingerlings at a prevalence rate 10% (Table 1). The parasite showed immature intra-erythrocytic gamont (Plate 2A) and meronts undergoing longitudinal binary fission (Plate 2B). While, in *O. niloticus* broodstocks, *Haemogregarina sp.* was observed in both blood samples and the blood within the gill tissue. Moreover, encysted metacerceria was recorded in gill tissue of the broodstocks.

*Histopatholgical examination*

The microscopical examination of gills revealed approximately 40-50% of the gill spaces and interstitium are expanded by large, multifocal to coalescing areas of abundant eosinophilic cellular and karyorrhectic debris (necrosis) along with large numbers of macrophages, lymphocytes, and degenerate neutrophils, abundant fibrin and edema (Plate 3A), with occasional hemorrhage. Additionally, within the gills there is severe inflammatory exudation and epithelial hyperplasia forming nodules with occasional different types of parasites, which may attach or occlude lamellar troughs. Frequently, the blood vessels and capillaries of primary and secondary lamellae are dilated and congested. Multifocally, primary lamellae are expanded 2-4X normal, secondary lamellae are fused, and lost or obscured by numerous foamy macrophages, fewer lymphocytes, and scattered hemorrhage admixed with necrotic debris. The protozoal cells of *Trichodina sp*. are seen attached to the apical region of secondary lamellae; irregularly round to oval, have a thin basophilic wall, and are filled with few eosinophilic oval spores (Plate 3B). Also, the fluke of *Gyrodactylus sp.* was seen adhered to gills of fry (Plate 3C). Furthermore, elongated intraerythrocytic stage of *Haemogregarina sp.* with displaced cell nuclei and abnormal morphology of infected

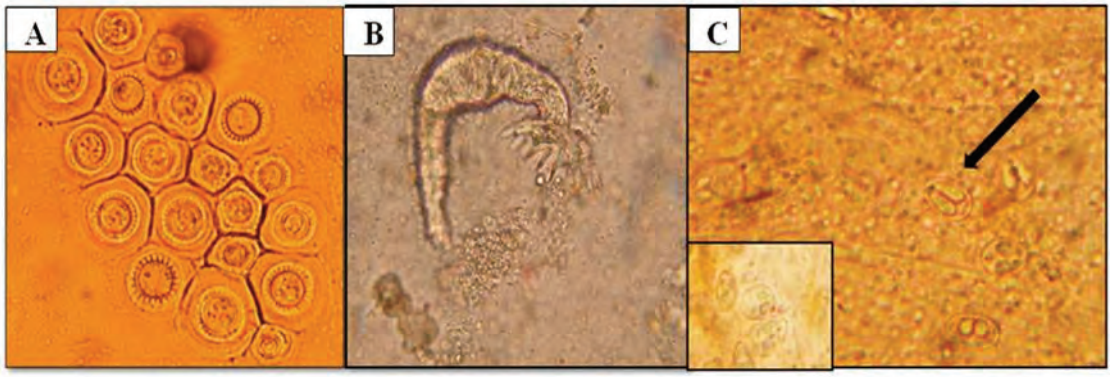


Plate 1. Wet mount preparation from *O. niloticus* showed A) heavy infestation with Trichodina sp from gills (x10), (B) *Gyrodactylus sp.* from gills (x10). C) *Myxosporean sp.* from kidneys appeared oval or rounded contains two polar capsules with transparent sporoplasm (X40).

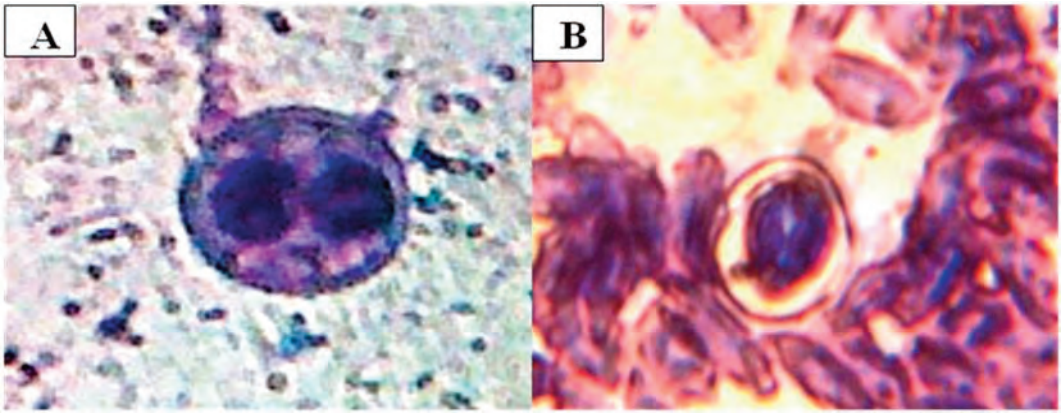


Plate 2. Blood film from fingerlings of *O. niloticus* showed A) immature intraerythrocytic gamont of *haemogregarina sp.* (Giemsa stain, x40). (B) Erythrocytes with meronts stage of *Haemogregarina sp.* undergoing longitudinal binary fission (Giemsa stain, x40).

erythrocytes was recorded in the blood cells of gills tissue of broodstocks (Plate 3D).

Rarely, developmental stages of myxosporea parasites are found scattered in the interstitial tissue and stroma of gill lamellae, contain one or more daughter cells (Plate 4A), the parasite was surrounded by macrophages and lymphocytes. The fully formed spores are ovoid, basophilic, with indistinguishable valves and two spherical polar capsules. Additionally, severe congestion of gills submucosal blood vessels with perivascular hemorrhage and accumulation of eosinophilic exudates

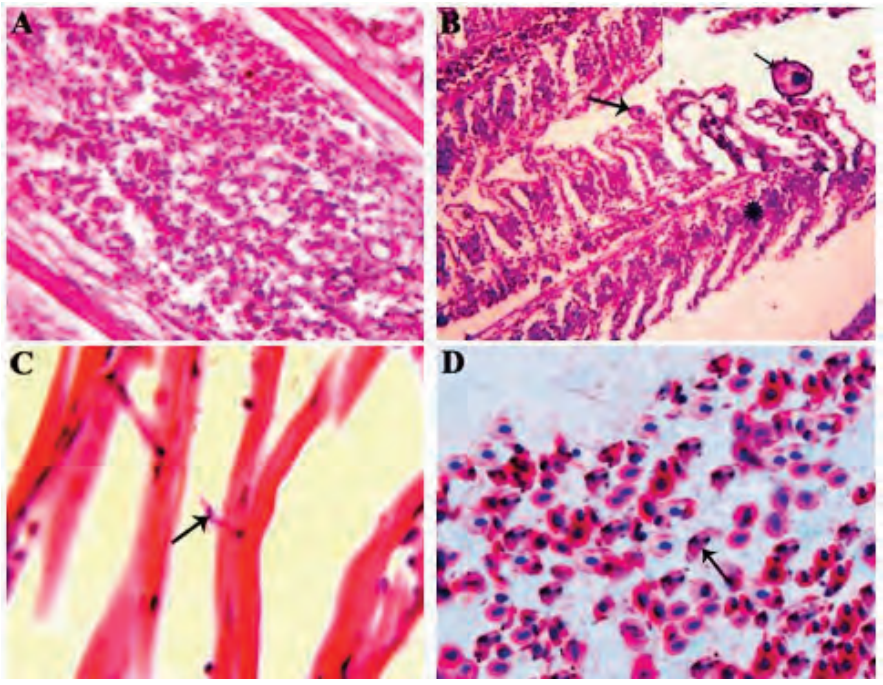


Plate 3. Hisopathological changes in infested gills of *O. niloticus* showed A) Entire absence of secondary lamellae with accumulation of eosinophilic inflammatory exudates admixed with inflammatory cells, (H&E, x400), B) Hyperplasia of secondary lamellae (astrik) in association with clubbing of the lamellae. Notice also adherence of trichodina to the tips of the lamellae (arrow), (H&E, x100, x1000). C) *Gyrodactylus sp.* adhered to gills of fry (arrow), (H&E, x1000), D) Intraerythrocytic developmental stage of haemogregarine (arrow), (Giemsa stain, x1000).

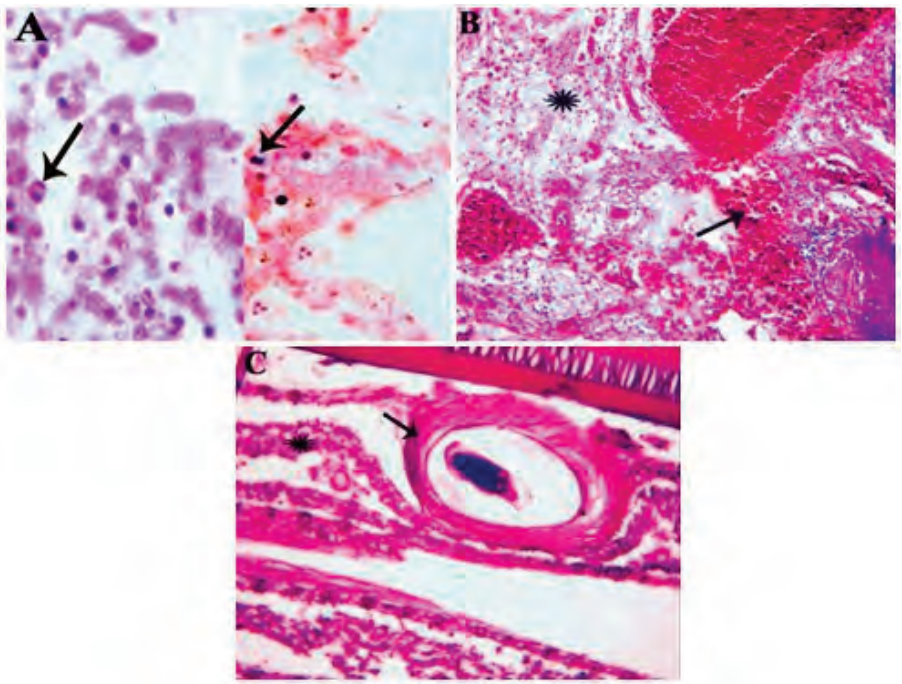


Plate 4. Histopathological changes of gill tissue in *O. niloticus* showed A) *Myxosporidian* spores adhered to the secondary gill lamellae (arrow), (Giemsa stain, x1000), B) Severe congestion of submucosal blood vessels with perivascular hemorrhage (arrow), with accumulation of eosinophilic exudates, (H&E, x200). C) Encysted metacercarea surrounded by fibrous connective tissue capsule (arrow) in combination with destruction of secondary lamellae (astrik), (H&E, x400).

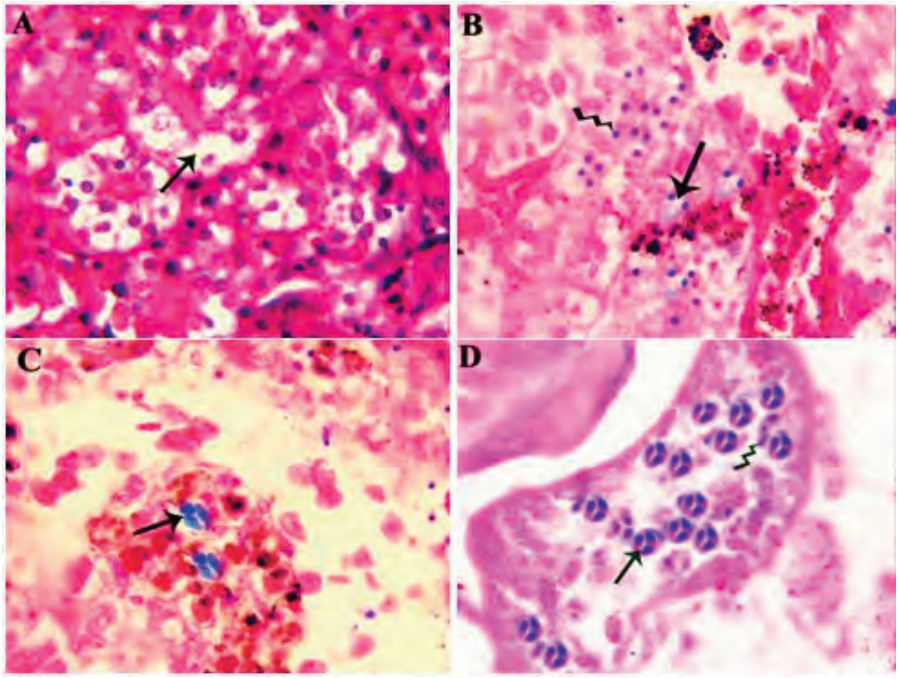


Plate 5. Histopathology changes of kidneys of *O. niloticus* showed A) Degeneration of the lining epithelium of renal tubules, (H&E, x400), B) Sporogenic stages of myxosporean parasites with two small spherical polar capsules (arrow) within the epithelial cells of the renal tubules. Notice also, numerous daughter cells in the lumen of some renal tubules (zigzag arrow), (Giemsa stain, x1000), C) Sporogenic stages of myxosporean parasites with three small spherical polar capsule (arrow) within the epithelial cells of the renal tubules in association with activation of melanomacrophage cells, (Giemsa stain, x1000), D) Sporogenic stages of myxosporean parasites (arrow) within the epithelial cells of the renal tubules of fry. Notice also, numerous daughter cells in the lumen of some renal tubules (zigzag arrow), (Giemsa stain, x1000).

was also demonstrated in the submucosa (Plate 4B). Occasionally, intralamellar encysted metacercariae are detected invading the gill tissue and encapsulated by sheet of connective tissue. Secondary lamellae are expanded by up to 4x normal by parasitic cysts. There is destruction of secondary lamellae with distortion of the normal gill architecture of broodstocks (Plate 4C).

Renal tissues of *O. niloticus* infested with myxosporidian revealed degenerative and necrotic changes in the lining epithelium of renal tubules (Plate 5A). The necrotic epithelium was shrunken with hypereosinophilic cytoplasm with pyknotic nucleus. Fully formed Sporogenic stages of *Myxosporean* spores were ovoid with indistinguishable valves and two (Plate 5B) or three spherical polar capsules at the anterior end were demonstrated in the lumen of renal tubules as well as in the interstitial tissues in association with activation of melanomacrophage cells (Plate 5C). Also, ovoid *Myxosporean* spores with three spherical polar capsules in combination with multiple daughter cells of myxosporidian were seen in the renal tubules of fry (Plate 5D). The polar bodies are densely stained than the sporoplasm.

## Discussion

Parasitic diseases have received a significant attention in Egypt due to its inverse impacts on fish hatcheries and farms causing severe economic losses especially in the early life stages (Eissa *et al*., 2000).

In the present study, *Trichodina sp.* was recorded from all examined fry, fingerlings, and broodstocks with a prevalence rate 100%. The intensity of the parasite was high (27-35 parasites/ microscopic field) among fry and fingerlings, while broodstocks showed low intensity (15-25 parasites/ microscopic field). Eissa (2002) stated that more than 25 parasites/ microscopic field in young fish was considered heavily infected and should be treated, while in adult, more than 50 parasites/ microscopic field should be observed, since the parasite was ectocommensal. The high mortalities of fry and fingerlings *O. niloticus* might be due to the severe hyperplasia and hypertrophy of the lining epithelial cells of the gill filaments with fusion of secondary lamellae, induced by the adhesive disc of the parasite, and consequently decreases the respiratory surface and gaseous exchange resulting in hypoxia (Yemmen *et al*., 2011). On other hand, Al-

varez-Pellitero (2008) stated that, parasites greatly affect the innate immunity of fish which modulated by pathogen recognizing receptors found on the gills that can limit the parasite load and the adaptive immunity demonstrated by the presence of T and B cells. However, fry and fingerlings showed immature innate and adaptive immunity, which can directly influence the infestation intensity, and hence fish become more susceptible to pathogen (Anderson, 1974). This might confirme the high mortalities observed among fry and fingerlings with no mortality among broodstocks due to the development of immune-system (Uribe *et al*., 2011). Similarly, Uzbilek and Yilidiz (2002) recorded that *Trichodina sp* causes 70% mortality among fry of cultured grass carp. In addition, Khan (2009) noted that, *Trichodina jadranica* was responsible for mass mortality in cultured fry and fingerlings of Atlantic cod (*Gadus morhua*). This indicates that mortalities due to trichodinasis were much higher in young fish than adult (Noga, 2000). The obtained results revealed that highest infestation with *Trichodina sp.* occurred in summer may be due high temperature which accelerates the reproduction and spread of the parasite (Kristmundsson *et al*., 2006). Ellis *et al*. (2002) stated that *Trichodina sp.* are considered ubiquitous ectoprotozoans, but the parasitic loads increased due to bad management practices such as high stocking densities and bad water quality.

*Gyrodactylus sp*. was observed in gills of fry, fingerlings and broodstocks at infestation rate 5, 12 and 10%, respectively. This indicates that prevalence of *Gyrodactylus sp.* is strongly increased with increasing host size and age (Raeymaekers *et al*., 2008). Moreover, García-Vásquez *et al*. (2007) recorded high mortalities in *Nile tilapia* fry infested with *Gyrodactylus cichlidaru*. The parasite could be transfer to new hosts via contact with infested fish, detached parasites drifting in the water column, or parasites attached to the substrate (Bakke *et al*., 1992). A very interesting fact that heavy infestation with a peritrichous ciliate, *Trichodina*, is commonly associated with monogenetic trematodes on the gill surfaces suggesting synergistic action between the two parasites causing mass mortalities (Pearse, 1972). High water temperature increases the rate of transmission of *Gyrodactylus* salaris due to increased parasite and/or host activity (Bakke *et al*., 1991). Moreover, stress (high water temperature) was accompanied by decreases of circulating lymphocytes, increase of macrophage cells, and enhanced red blood cell degradation resulting in increases the susceptibility of fish to diseases (Peters and Schwarzen, 1985). This could support the results of the current study in which high mortalities occurred among *O. niloticus* fry and fingerlings in summer.

Regarding to *Myxosporean* spores identified in kidneys and gills of *O. niloticus* at the different life stages, the infestation rate was 100%. The infestation of *O. niloticus* broodstocks with myxsporean sp could occur via ingestion of infested oligochahetes containing the infective actinosporean stage (Lom and Dykova, 1995) or may be through direct contact with mature actinospores that released from their alternative hosts (oligochahetes) and swimming free in the water column (El-Matbouli and Hoffmann, 1998). The actinosporean stage was able to survive from 4 to 25 days in water column, depending on temperature and species (Xiao and Desser, 2000). Moreover, *myxoporean sp.* parasitize fish gonads (Reed *et al*., 2003), consequently the infected gametes released during spawning which may be transmitted to the offspring (Sitja`-Bobadilla, 2009). Meanwhile, vertical transmission has not recorded for *myxoporean sp.* yet.

Histopathology of gills infected with *Myxosporean sp.* showed severe congestion of submucosal blood vessels and perivascular hemorrhage with accumulation of fibrinous exudates admixed with leukocytes with presence of myxospore was seen. This observation correlates with the histopathological changes recorded by Abdel-Latif (2007) on gills of *Clarias gariepinus* infested with *Henneguya sp.* Similarly, Madhavan *et al*. (2013) found plasmodia cyst of *Myxobolus sp.* attached on the gill lamellae of minor carps, *Labeo calbasu*. Furthermore, kidneys of infected *O. niloticus* showed degenerative and necrotic changes in the lining epithelium of renal tubules with numerous extra-sporogenic stages and sporogenic stages of myxosporidian parasites in association with activation of melanomacrophage. This finding was similarly to Jones *et al*. (2004) and Sorour (2008). Myxosporean fish parasites are capable of producing proteolytic enzymes responsible for tissue deterioration and possibly parasite encystation (Martone *et al*., 1999). The activation of melanomacrophage centers was of vital importance in the development of the immune response to parasite (Roberts, 2001), which responsible for deposition

of resistant pathogens such as parasitic spores (Roberts, 1975) and antigen processing in immune responses (Agius, 1985). The degenerative changes of gills and kidneys resulted in hypoxia and osmoregulatory failure and consequently death of host. The degree of pathogenicity depends on many factors, such as the *Myxosporean sp*ecies involved, its life cycle and biology, the host species, host age, state of nutrition and host resistance.

*Haemogregarina sp.* was found in the blood of fingerlings and gill tissue of broodstocks. These results come in agreement with Siddall and Desser, (1993) and Adam *et al*. (2009) they recorded different *Haemogregarina sp.* from freshwater fishes. It was known that Haematophagous leeches and isopods are considered as vectors for marine haemogregarines (Davies *et al*., 2004). *O. niloticus* broodstocks could be infested through infested leech biting, while, fingerlings could be infested with *Haemogregarina sp.* orally through ingestion of freshwater isopoda. This supported by Davies and Johnston (1976) and Davies and Smit (2001) as they reported that ingestion of *Gnathiids* is opoda were proposed as vectors for *Haemogregarine bigemina* in juvenile fish less than 4 cm that could not have been exposed to leeches. Vertical transmission of *Haemogregarina sp.* from adult to young fishes is improbable (Davies *et al*., 2004).

In *O. niloticus* broodstocks, gill tissues infested with *Haemogregarina sp.* revealed erythrocytic vacuolation, intraerythrocytic elongated haemogregarine parasites with displaced cell nuclei. This result similarly to finding (Siddal and Desser, 1993), who recorded that infected erythrocytes with *Haemogregarina myoxocephali* appeared vacuolated and exhibited shrinkage and altered shape. In the current study, *Haemogregarina sp.* infecting blood cells are clearly observed in Giemsa stained tissue sections that confirm heavy infections with this parasite. These findings confirm the findings of Bruno *et al*. (2006), who reported that *Haemogregarina sp.* may be detectable in blood cells in heavily infected tissue sections.

Encysted metacercarea surrounded by fibrous connective tissue capsule in association with destruction of secondary lamellae with distortion of the normal gill architecture was observed in *O. niloticus* broodstocks. This result was similarly to Abdel-Latif (2007), Reda *et al*. (2010) and Eissa *et al*. (2011), who recorded congestion of central blood vessels and mononuclear inflammatory cellular infiltration in gills of infested *O. niloticus* with encysted metacerceria. Also, Shoaibi Omrani *et al*. (2010) recorded cartilage proliferation, hyperplasia, hypertrophy and fusion of gill filament in platyfish infested with metacerceria. There were no encysted metacerceria in fry or fingerlings. This may be due to stocking fry *O. niloticus* after hatching in concert pond where there is no contact with snails (1st intermediate host) and final host (birds) as in earthen ponds where the broodstocks reared. This finding was supported by Reda *et al*. (2010) who stated that highest infestation with encysted metacerceria of gills *O. niloticus* was recorded in adult stage.

## Conclusion

Fry and fingerlings stages were more susceptible to parasitic infestation than broodstocks showing high mortality rate in hatcheries. These parasites become a potential threat to host inducing immune-suppression and hence favoring the entrance and establishment of other pathogens. Therefore, we recommended following semi-artificial propagation to avoid parasitic diseases that may be transmitted from broodstocks to the hatched fry by long contact.

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