# Field studies on Prevailing Internal Parasitic Diseases in Male and hybrid tilapia relation to Monosex Tilapia at Kafr El-Sheikh Governorate Fish Farms

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**Abstract:** The present study was carried out on 1800 specimens of *Oreochromis niloticus* (*phenotypic , hybrids and monosex* of different lengths and body weights. They were randomly collected at different seasons from Kafr El-Sheikh Governorate cultured fish farms. The clinical signs of infested fish revealed no pathognomonic abnormalities on the external body surface .Such fish were shown emaciation. The postmortem showed that the internal organs were appeared anemic with enlargement and congestion. As well as, haemorrhage and ulceration of intestine and stomach mucous membrane. Monogenetic trematode (*Enterogyrus* cichlidarum*),* Adult flukes including *(Orientocreadium batrochoides, Afromacroderoides sp, Astiotrema reniferum* and *Eumasenia egypticus*), Nematodes including*( Procamallanas laeviconchus* and *Paracamallanas cyathopharynx),* Cestodes including *Polyonchobothrium sp and* Acanthocephalan including *Acanthocentis tilapiae* were investigated and identified. The highest prevalence possessed in hybrids of *O. niloticus* while monosex *O.niloticus* occupied the last position.. Also some physico-chemical parameters of pond waters represented as alkalinity pH, salinity, ammonia and sulphates were examined in relation to the infestation rate with internal parasites.

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**Keywords:** Internal parasites, *O.niloticus, Hybrids, monosex* and physico-chemical parameters.

## 1. Introduction

Internal parasitic diseases have the upper hand in fish parasitic diseases regarding the low body gain, high mortality. In addition, such diseases lead to gastrointestinal abrasions which facilitate the invasion of the opportunistic microorganisms. Where unfavourable environmental conditions contribute to stress which was weakens immunity and opens the pathway to pathogens (Kabata, 1985) and (Eissa, 2002). The clinical picture of infested fish revealed no pathognomonic abnormalities on the external body surface .Such fish were shown emaciation. The postmortem showed that the internal organs were appeared anemic with enlargement and congestion Bassiony (2000) and Ibtsam (2004). In addation, Abd El Hady (1998) recorded presence of *Eumasenia aegyptiacus, Orientocadium batrchoides, Astiotrema reiferum* and *Afromacroids sp* from *Tilapia sp*. The highest prevalence of *Enterogyrus* was in summer by autumn then followed by spring and the lowest rate of infestation was in winter Osman (2005). Nadia Mahfouz (1991) recorded *Paracamallanus cyathopharynx* with a rate of 1.4 % in *Tilapia sp* while *Procamallanus laeviconchus* 2.8% in *Tilapia sp* at Edfina and Barseek fish farms .The prevalence of nematodes were 9.25, 27.27, 2.65 and 0.0% in winter, spring, summer and autumn respectively. Omoregie *et al*. (1995) recorded that d *Polyonchobthrium sp* in *Oreochromus niloticus* from Panyam fish farm, Nigeria. Tawfik (2005) recorded that *Acanthosentis tilapiae* isolated from *Oreochromis niloticus* at Abbassa fish farms. The present investigation was planned to studying the clinical signs of the examined affected fish isolation and identification of the internal parasites infesting cultured male phenotypic, hybrids and monosex *O.niloticus* in kafr El-Sheikh fish farms, analysis of some water hydrochemistery of affected aquacultures, Studying the relationship between the seasonal prevalence of parasitic diseases and the water quality and investigation of histopathological alterations of infested male phenotypic, hybrids and monosex *O. niloticus*.

## 2. Material and Methods

**Fish specimens:**

A total number of 1800 cultured *Oreochromis niloticus (O.niloticus)* of various life stages; fry, fingerling and adult Tilapia of different Male fish types (phenotypic, hybrids and hormonally treated monosex were collected from special fish farms in Kafr El Sheikh Governorate. The length of fry, fingerlings and adult specimens were ranged from 1- 1.5, 2 - 8 and 20 - 30 cm .While as their body

weights were ranged from 0.9 – 15.0, 17 – 28.7 and 105 – 220 g respectively.

**Water samples:**

A clean 48 water sample flasks, one litre volume were equipped with a cork stopper. The flasks were rinsed several times with distilled water and sterilized in a hot air oven at 180 C° for one hour.

**Clinical examination:**

The collected fish types were examined clinically for clinical signs and P.M. lesions according to the methods described by Noga (2010).

**Parasitological examination:**

The alimentary canal of each fish was separated, dissected and divided into three main parts: fore, mid and hindgut. Each part was washed with physiological saline for several times to get rid of mucus and coarse particles that may be adherent to the parasites, then each part was opened and examined in a Petri dish under binocular dissecting microscope. The parasites were collected by Pasteur pipette and dissecting needle and transferred into Petri dishes containing warm saline solution was obtaining a fully relaxed and extended parasites.

**Preparation of permanent samples**

The collected helminthes (trematodes, cestodes and acanthocephalan) were left overnight in refrigerator to allow the worm to die; then compressed gently between two glass slides and fixed in 4 % formalin. The worms were washed in running water then soaked in alum carmine for 3 hrs. After staining; worms were washed in distilled water and passed through ascending grades of ethyl alcohol 50, 70, 90 and 100%, then transferred into xylol and clove oil respectively. Finally mounted with Canda balsam and covered with cover slide. Then slides were incubated at 60ºC for 24 hrs to driving of the air bubbles (Kabata, 1985). The collected nematodes were washed in saline then relaxed in refrigerator at 4C° and then immersed in hot alcohol-glycerin mixture until all alcohol was evaporated and the specimens remained in absolute glycerin. Worms was cleared in lactophenol and mounted in glycerin-gelatin according to Schmidely (1993).

**Identification of parasites:**

The helminthic parasites were morphologically and parasitologically identified according to Yamaguti (1985) .

**Physico – chemical analysis of water:**

pH value of water was measured at the different locations in the fish pond by means of a digital pH meter (Ph CP. Hanna instruments. Italy). Total salinity was estimated by DR 2010 (at wave length 530, programs 88),also ammonia (mg /l) was measured at wave length 655, programs 342). Alkalinity was tested using (Chest own Maryland) alkalinity test kits 21620.Sulphate was measured , using HACH reagent, code 4630 in HACH apparatus, model CC. PS, (Adams 1990).

**3. Results**

**Clinical examination:**

The infested fishes showed signs of emaciation with sunken eyes and petechial haemorrhage on the surface of abdomen and intestinal wall was congested with the presence of ulcer and protruded from anus accompanied with large amount of catarrhal mucoid secretion. Also, they revealed internal organs of naturally infested fish were pale, aneamic with enlargement and congestion of spleen, liver with distended gallbladder, enteritis, haemorrhage and ulceration of stomach as well as intestinal mucous membrane. Some fishes showed slight bulging of the stomach, congestion and haemorrhage on the mucous membrane with watery food especially in heavily infested cases. White nodules in posterior kidney (plate, 1).

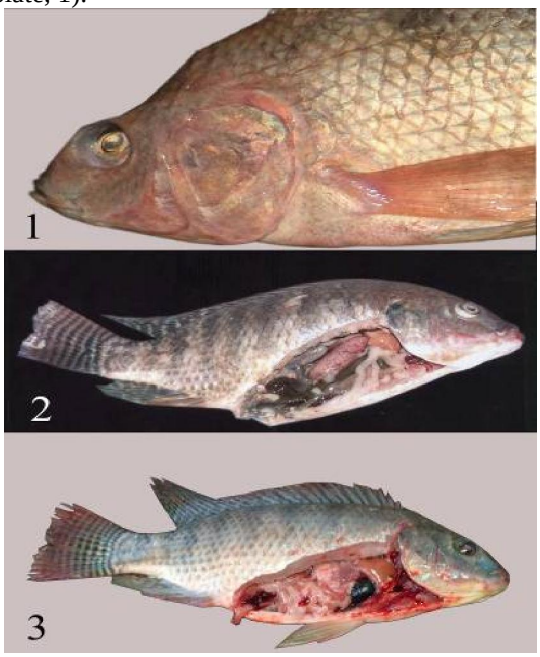


**Plate (1):1-** Hybrid *Oreochromis niloticus* with sinking eye. **2-** Hybrid *Oreochromis niloticus* with slightly turgid stomach and inflammation of the intestines.**3-** Monosex *Oreochromis niloticus* showing distended of gall bladder

**Parasitological examination:**

Adult worms were isolated form the stomach of infested fish. Such adult worms identified as *Enterogyrus cichlidarum* and adult worms were isolated from midgut of infested fish. Identified as *Orientocreadium batrochoides and Afromacroderoides sp.* Also, adult worms were collected from foregut of infested fish identified as *Astiotrema reniferum, Eumasenia egypticus, Procamallanus laeviconchus and Paracamallanus cyathopharynx***.** While, thorny-head worms were isolated from hindgut of intestine of infested Tilapia sp identified as *Acanthosentis tilapiae* (plate2).

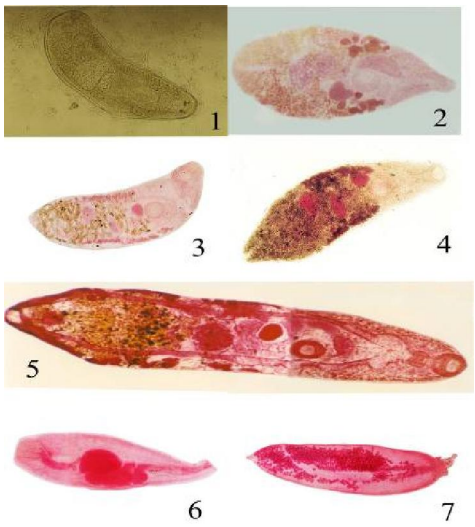


**Plate (2): 1-** *Enterogyrus cichlidarum* from stomach of hybrid *Oreochromis niloticus*. Wet mount. X 200.**2-** Adult fluke, *Eumasenia aegypticus*. Stain: Acetic acid alum Carmine X 4.**3-** Adult fluke, *Afromacroderoides sp*. Stain: Acetic acid alum carmine X 4.**4-** Adult fluke, *Astiotrema reniferum*. Stain: Acetic acid alum Carmine X 4.**5-** Adult fluke, *Orientocreadium batrochoides*. Soalted from midgut of *O. niloticus*. Stain: Acetic acid alum Carmine X 4.**6-** Adult fluke, *Acanthocentis male*. Stain: Acetic acid alum Carmine X 4. **7-** Adult fluke, *Acanthocentis female*. Stain: Acetic acid alum CarmineX4.

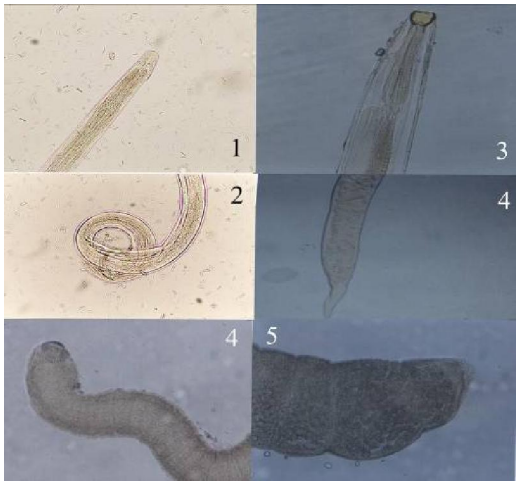


**Plate (3): 1-** *Procamallanus laeviconchus*. (Anterior end).Wet mount X10. **2-** *Procamallanus eviconchus*. (Posterior end). Wet mount X 10.**3-** *Paracamallanus cyathopharynx*. (Anterior end).Wet mount X10. **4-** *Paracamallanus cyathopharynx*. (Posterior end). Wet mount X 10. **4-** *Polyonchobothrium sp.* (Anterior end).Wet mount X 10. **5-** *Polyonchobothrium sp*. (Posterior end). Wet mount X 10.

**Prevalence and distribution of helminthes:**

Internal parasitic infestations of phenotypic, hybrids and monosex *O. niloticus* with *Enterogyrus cichlidarum Orientocreadium batrochoides Afromacroderoides sp Astiotrema reniferum , Eumasenia egypticus , Procamallanus laeviconchus and Paracamallanus cyathopharynx and Acanthosentis tilapiae* were recorded in Table (1 and 2).

**Water analysis and seasonal prevalance:**

The prevalence of parasitic infestation with different water parameters were recorded in Table (3)

**Table ( 1 ): Prevalence of parasitic infestations among males of phenotypic, hybrid and monosex *O.niloticus*.**

| Tilapia | No, of exam. fish | Internal parasites | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Adult trematodes | | Nematodes | | Cestode | | Acanthocephala | |
| | | N. | % | NO | % | NO. | % | NO | % |
| Male phenotypic *O. niloticus*. | 600 | 37 | 6.2 | 11 | 1.8 | 2 | 0.3 | 21 | 3.5 |
| Male hybrids *O. niloticus*. | 600 | 66 | 11 | 17 | 2.8 | 4 | 0.6 | 31 | 5.2 |
| Male monosex *O. niloticus*. | 600 | 27 | 4.5 | 5 | 0.9 | 0 | 0 | 9 | 1.5 |
| Total | 1800 | 130 | 21.7 | 33 | 5.5 | 6 | 1 | 61 | 10.2 |

**Table (2 ):Distribution of helminthes in different types of Tilapias.**

| Site of infection | Class of parasites | Genus of parasites | Phenotypic *O. niloticus* | | Hybrids of *O. niloticus*. | | Monosex *O. niloticus* | |
|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | No. | % |
| Stomach | Trematodes | ***Enterogyrus cichlidarum*** | 2 | 0.3 | 3 | 0.5 | 1 | 0.2 |
| | Nematodes. | ***Procamallanus laeviconchus*** | 6 | 1 | 8 | 1.3 | 3 | 0.5 |
| | | ***Paracamallanus cyathopharynx*** | 5 | 0.8 | 9 | 1.5 | 2 | 0.4 |
| Intestine | Trematodes | **Adult trematodes** | 7 | 1.2 | 13 | 2.2 | 0 | 0 |
| | Cestodes | ***Polyonchobothrium sp*** | 2 | 0.3 | 4 | 0.6 | 0 | 0 |
| | Acanthocephala | ***Acanthocentis tilapiae*** | 21 | 3.5 | 31 | 5.2 | 9 | 1.5 |

**Table ( 3 ): The seasonal correlations between the average of some water parameters in different fish cultures with Endo parasitic infestations.**

| Types of Tilapia culture | No of Exam. fishes | No of Infested. fishes | Types of parasites | seasons | | | | Seasons | Salinity ppt | pH | ammonia mg/L | D o | Alkalinity ppt | Sulphate ppt |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Spring | Summer | Autumn | Winter | | | | | | | |
| **Male phenotypic** ***O.niloticu s.*** | 600 | 202 | Trematodes | 1.3 | 1.3 | 1.2 | 2.3 | sp | 2 | 7.2 | 0.3 | 8 | 210 | 90 |
| | | | Cestode | 0 | 0.3 | 0 | 0 | su | 1.3 | 6.8 | 0.4 | 6 | 180 | 50 |
| | | | Nematodes | 0.3 | 0.7 | 0.8 | 0 | Aut. | 1..2 | 6.2 | 0..3 | 8 | 190 | 53 |
| | | | Acanthocephala | 1 | 1.2 | 0.5 | 0.8 | Win. | 2 | 7.5 | 0.2 | 10 | 215 | 110 |
| | | | Trematodes | 1.5 | 1.8 | 3.2 | 4.5 | sp | 2 | 7.2 | 0.3 | 8 | 210 | 90 |
| **Male hybrids of** ***O. niloticus*** | 600 | 302 | Cestode | 0 | 0.3 | 0.3 | 0 | su | 1.3 | 6.8 | 0.4 | 6 | 180 | 55 |
| | | | Nematodes | 0.8 | 1 | 0.5 | 0.5 | Aut. | 1.7 | 6.3 | 0..2 | 8 | 193 | 52 |
| | | | Acanthocephala | 0.8 | 1 | 1.7 | 1.7 | Win. | 2 | 7.6 | 0.1 | 10 | 209 | 115 |
| **Male Monosex** ***O. niloticus.*** | 600 | 96 | Trematodes | 0.7 | 3 | 0.8 | 0 | sp | 1.0 | 7.5 | 0.1 | 10 | 160 | 50 |
| | | | Nematodes | 0.3 | 0.5 | 0 | 0 | su | 1.2 | 6.4 | 0.3 | 12 | 170 | 50 |
| | | | Cestode | 0 | 0 | 0 | 0 | Aut. | 1..2 | 6.2 | 0.2 | 8 | 173 | 41 |
| | | | Acanthocephala | 0.3 | 0.7 | 0.5 | 0 | Win. | 1.0 | 7.1 | 0.1 | 10 | 210 | 93 |

## 4. Discussions

The present study deals with most of different internal parasitic diseases among naturally infested the cultured *O. niloticus* (phenotypic , hybrid and monosex ) in relation to the seasonal prevalence and water parameters in Kafr El- Sheikh fish farms. The internal organs of naturally infested fish appeared pale, anemic with enlargement and congestion of spleen, liver with distended gallbladder. Signs of emaciation with petechial haemorrhage on the surface of abdomen and slight bulging of stomach was obseved. While, intestinal wall was congested with the presence of ulcer and protruded from anus accompanied with large amount of catarrhal mucoid secretion. This clinical picture nearly was similar to that recorded by Osman (2005). This picture may be explained due to the presence of Enterogyrus, nematodes, cestodes and thorny headed worms which cause harmful effect as they embedded themselves between the villi of intestine causing local damage to the intestinal mucosa and possibly peritonitis. Proteolytic enzymes may be discharged from some adult worms degrading the intestinal tissues (Woo, 1995). Regarding the internal monogenea (*Enterogyrus cichlidarum)* was morphologically and parasitologically described and was nearly similar to the descriptions given by Khidr (1996). Concerning to the cestodes (*Polyonchobothrium sp*) was isolated and identified from infested *Tilapia sp.* Such identification is nearly similar that recorded by Yamaguti (1985). Regarding to the isolated nematodes from naturally infested Tilapia fishes, isolation and identification of *Procamallanus laeviconchus* and *Paracamallanus cyathopharynx* were undertaken that nearly similar to those of original descriptions by Woo (1995). Finally, morphological and parasitological examinations of Tilapia fishes revealed isolation and identification of *Acanthosentis tilapiae* whose descriptions are nearly similar to those of original description by Yamaguti, (1985).

In the present study a total prevalence of Enterogyrosis in phenotypic, hybrid and monosex was 0.3, 0.5, and 0.2% respectively. Such results are lower than recorded by Eid and Negm, (1987) who reported that the prevalence of *Enterogyrus cichlidarum* from *O.niloticus* collected from (Bahr Mouise) was 13.3%, Ibtsam (2004) who recorded

60% of Entergyrosis. Also, disagree with that recorded by Osman (2005) who found a prevalence of Entergyrosis as 67.2%. These variations may be attributed to the water quality criteria and age of fish as such worms are stomach flukes need aged fish have well developed stomach and its wall was thicker for adaptation and fixation.

Regarding the total prevalence of adult flukes in phenotypic, hybrid and monosex were 1.2, 2.2, 0 % respectively. These results disagreed with the finding of Hassan (1992) who recorded the peak of adult trematodes in *O. niloticus* were 6%. Such result was lower than recorded by Abd El- Hady (1998) who recorded that a prevalence of adult flukes was 15.58 %.

Concerning the total prevalence of *Procamallanus laeviconchus* was 1.8, 2.8 and 0.9% from phenotypic, hybrid and monosex respectively. These findings nearly agree with that met by Nadia Mahfouz (1991) who recorded a prevalence of *P. laeviconchus* as 2.8% in *O.niloticus* and lower than that recorded by El- Naffer *et al.* (1983) who recorded a prevalence of *P.laeviconchus* was in *Tilapia sp* 39%. While in *Paracamallanus cyathopharynx* were 0.8, 1.5, and 0.4%. These finding nearly agree with that recorded Abd El-Wahed (1992) who recorded a prevalence of P. *cyathopharynx* was 1.4% in *O.niloticus*. These results may be attributed to different types of fish, the presence of intermediate host (Snails), the suitable temperature which consider the main survival factors for these intermediate host and aquatic birds.

The highest infestation of phenotypic, hybrid and monosex were recorded in summer 3.5%, winter 4.4% and spring 2%. These results disagreed with that recorded by Nadia Mahfouz (1991) who recorded a prevalence of nematodes infestation in winter, spring, summer and autumn were 9.25, 27.27, 2.65 and 0% respectively. This result may be attributed to different types of fish, the presence of intermediate host (*Piscivorous*), and the suitable temperature which consider the main survival factors for these intermediate host and aquatic birds.

In this study, *Polyonchobothrium sp* could be detected with a prevalence 0.3, 0.6 and 0% from phenotypic and hybrid respectively. These findings are lower than that recorded by Hassan (1992) who found up to 7.5%.

The highest infestation of phenotypic, hybrid and monosex were recorded in summer 3.5% and winter 4.4% and spring 2%. These results disagreed with that recorded by Nadia Mahfouz (1991) who recorded a prevalence of nematodes infestation in winter, spring, summer and autumn were 9.25, 27.27, 2.65 and 0% respectively. These differences may be due to variation in climatic and ecological factors which affect on intermediate host copepods (Cyclops) and aquatic birds.

Finally, the prevalence of *Acanthosentis tilapiae* in cultured (phenotypic, hybrid and monosex) was 3.5, 5.2 and 1.5% respectively. These findings nearly agree with that recorded by Eissa *et al.* (1996) and Rawia Adawy (2000) who recorded a prevalence of *A. tilapiae* in cultured *Tilapia sp* as 2.4 and 3.7% respectively and lower than that recorded by Eid (1997) who recorded a prevalence of *A. tilapiae* in *Tilapia sp* as 37.8%.

Concerning the highest infestation in phenotypic, hybrid were recorded in summer, spring, winter and autumn as 5.8, 3, 1.4 and 0% respectively. These results nearly agreed with that recorded Rawia Adway (2000) a prevalence of *Acanthocentis tilapiae* in cultured *Tilapia sp* in summer as 4.4% and disagree with Bassiony (2002) who mentioned that the highest infestation rate a prevalence of *A. tilapiae* in cultured *Tilapia sp* was in summer, autumn, spring and winter seasons were 39, 24, 21, 16.2 and 17.4% respectively. Also, Ibtsam (2004) who mentioned a prevalence of *A. tilapiae* in cultured *Tilapia sp* as in summer, autumn, spring and winter seasons were 12, 10, 17.4 and 0% respectively and in Tawfik (2005) who mentioned that the highest infestation rate a prevalence of *A. tilapiae* in cultured *Tilapia sp* in summer and winter as 14 and 7% respectively. This result may be attributed to different type of fish the presence of intermediate host (amphipod and isopod), the suitable temperature which consider the main survival factors for these intermediate host.

In the present study, all of the water parameters namely pH, salinity, ammonia, alkalinity, DO and sulphates in cultured fish farms, were within the permissible limits throughout the period of study according to APHA (1985). The epizootiological point of view to be important, are the predisposing factors for the wide spread of parasites among different Tilapia fishes in this study.

There was a positive correlation between the pH and alkalinity in water and the prevalence of each of (*Protozoa, Monogenea* and *Acanthocephala*). Negative correlation with prevalence of each of (nematodes and cestodes). These may be attributed to the fact that increasing the pH and calcium carbonate in water of fish ponds represent a stress factor on respiration process of fish, especially the gills and may be facilitate such parasites to infect fish.

The negative correlation between the DO in water and the prevalence of each of *Acanthocephala* may be attributed to the fact that low DO represent a stress factor on respiration process fish, leading to asphyxia and loss of escape reflex leading to entry of fish parasites .The present study, revealed that the

levels of total ammonia and salinity in ponds containing monosex tilapias were within the normal levels. On the other side, there was increase of both parameters in ponds of hybrids and phenotypic. This may be due to receiving agricultural drainage, domestic sewage and misuse of poultry dropping (Sabla) that revealed the poor water quality in this location (ponds) which was accompanied by increase the prevalence of *Protozoa, Ergasilus sp* and trematodes infection. These results agree with that recorded by Naguib and Abu Essa (1999) who found that, the incidence of the encysted metacercariae in muscles of infected fish during the breeding season was 56.2% in fish farms received the agricultural drainage. There is a positively correlation between water sulphate levels and the prevalence of *Acanthosentis tilapiae.* It may be due to limiting factor affecting algal growth (Elewa and Mahdy., 1988).

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