



The inter-relationship of water quality parameters and fish parasite occurrence

Karam I. Ashmawy, Fatma A. Hiekal, Somaia S. Abo-Akadda, Nadia E. Laban
Parasitology Department, Faculty of Veterinary Medicine, Alexandria University

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*Correspondence to:

labn.nadia@yahoo.com

ABSTRACT

This study investigated the composition and structure of parasitic communities of *Oreochromis niloticus* with respect to levels of water quality in three ecosystems. A total of 360 *O. niloticus* were examined from three water courses. Idku lake (moderately polluted), Edfina; Natural Nile resources (polluted) and Mariout lake (severely polluted). Water samples were collected for determination of some water quality parameters and the concentration of heavy metals. Trematodes, monogenes, protozoa, crustacea and acanthocephalan were found in all sample except Mariout lake where Acanthocephala and metacercaria of Clinostomum were completely absent. Ciliated protozoa predominated the parasitic infestation. The results also indicated that there was an inverse relationship between fish parasites communities and water quality parameters.

1. INTRODUCTION

Fish is one of our most valuable sources of protein food. Worldwide, people obtain about 25% of their animal protein from fish and shell fish. Majority of fish serve as an intermediate host for many parasites which reduces the food value of fish and cause mass mortality (Galli et al., 2001; Bunkley-Williams et al., 2006). About 80% of fish diseases are parasitic especially in warm water fish. (Eissa and Gharib, 2005) which affect fish causing retardation of growth especially for young fish and increasing the possibility of the secondary infections by decreasing the fish immunity (Ilan and lengy, 1963 and Elamei, 2001). Tilapia species are freshwater fish belonging to the family of Cichlidae and considered to be more resistant compared to other species of cultured fish (Bondad-Reantaso et al., 2005; Akoll et al., 2012).

In recent years there is increasing awareness that parasitism should be investigated in the light of respective environmental conditions (Mackenzie et al., 1995; Lafferty et al., 2004). Water pollution can effectively limit the occurrence of some species of fish parasites and affect their qualitative and quantitative composition through influencing their eggs, free living larval stages and their intermediate or final hosts (Popielek, 2001).

It becomes clear that host's environmental conditions are also extremely important for parasites although their effects may differ depending on parasite life cycles. For heteroxenous metazoan parasites environmental conditions must be favorable for all host levels (intermediate and final hosts) and for eventually free-living stages of the parasites (Mackenzie, 1999) whereas parasites with direct life cycle (monoxenous) are less affected Pérez-del Olmo et al., (2007). Also the composition and diversity of total parasite communities of a particular host (e.g. fish) were found to reflect the ecological condition of habitats where the host occurs. For example, Nachev and Sures (2009) and Chapman et al., (2015) reported higher parasite diversity at less polluted sampling sites, whereas the composition of the parasite fauna and the abundance of some parasites showed a clear relationship with the pollution gradient.

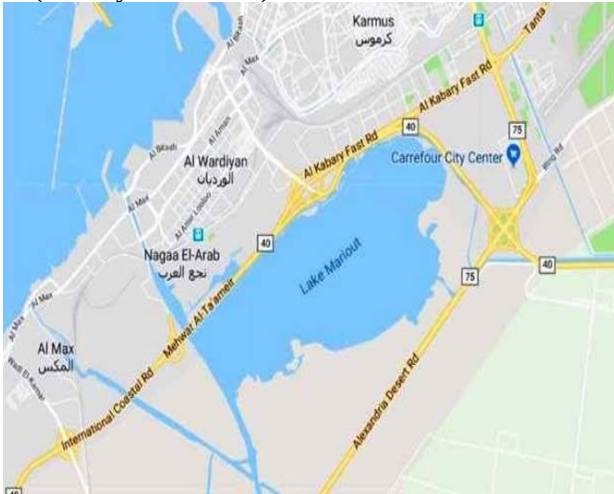
The present study was initiated to examine and compare the water quality parameters and parasitic load on *O. niloticus* fish investigated at three different ecosystems and to test the impact of parasites as quality indicator to pollution.

2. MATERIAL AND METHODS

2.1. Study area

2.1.1. Site 1(Lake Mariout)

Mariout Lake is one of the main fishing grounds of Alexandria located between longitude 29°47.1' to 29°50.4' E and latitude 31°7.5' to 31°9' N . It represents a shallow sheltered Estuary west of Alexandria, extends for about 15 km between El-Agamy headland to the west to the Western Harbor to the east and from the coast to a depth of about 30 m (Stanley et al, 2006)



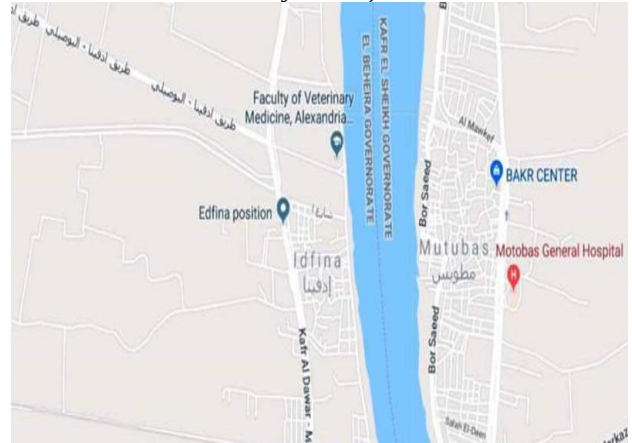
2.1. 2. Site 2 (Lake Idku)

Lake Idku is a shallow brackish coastal basin situated on the western margin of the Nile Delta, 30 km to the east of Alexandria with an area of about 126 km² (El-Shenawy, 1994). It lies west of the Rosetta branch of the River Nile, and extends from east to west for a distance of about 19 km, with a surface area of about 85 km² and an average depth of about 1 m. It is adjoining the Mediterranean coast at latitude 31_ 150 N and longitude 30_ 150 E. It has an average width of 6 km with an average depth of about one meter.



2.1.3. Site3 (Edfina (Natural Nile branch))

Rosetta River Nile Branch represents the main freshwater stream that extends northwards for about 236 km on the western boundary of the Nile Delta from Egypt's Delta Barrage. Rosetta branch has an average width of 180 m and depth from 2 to 4 m. The estuary is delimited by a barrage for controlling water discharge at Edfina City, 30 km upstream the sea. (El Gammal and El Shazely, 2008)



2.2. Fish samples

A total of 360 live fish specimens from *Tilapia* spp. were collected from natural Nile resources (Rashid branch) as well as from market of Edfina, from Idku Lake at Behera Province and from Mariout lake at Alexandria Province during the period of May 2017 to March 2018. Fishes were transported alive to the laboratory of parasitology, Faculty of Veterinary medicine, Alexandria university in large plastic bags filled with water from the water of the same resources. The fish samples were examined immediately

2.3. Water samples

The collected water samples from each site were transported to the Water pollution and Marine Environmental Lab, Alexandria University for water analysis. All samples were obtained in sterile plastic bottles and stored according to Standard Methods described by APHA (American Public Health Association) (2005) then chemically and heavy metals analyzed. The water quality assessed according to Anita and Pooja (2013). Heavy metals concentration were assessed according to Meade (1989) in the three investigated sites and the permissible limits were recommended by Egyptian Organization for Standardization (1993).

2.4. Measuring of chemical parameters:

The collected water samples were analyzed for major chemical water quality parameters such as; pH, COD (organic matter) and salinity. These parameters were measured by using water checker U-10 Horiba Ltd. Other parameters like; total alkalinity, nitrite, ammonia and total hardness were measured by

traditional manual methods according to (USEPA, 1983 and Eaton et al., 1995).

2.5. Determination of heavy metals:

The collected water samples were analyzed to determine the concentration of heavy metals (Cu, Cd and Pb) as process of Clescerl et al. (1999).

2.6. Examination of fish for External parasites

Each fish specimen was examined with naked eyes and hand-held lens, with special emphasis on fins, operculum nostrils, and buccal cavity. Gills after removal, were examined under a dissecting microscope smears scanned under a light microscope (40X and 100X objectives lens) and pieces of the gills were dipped in 4% formalin (vigorously shaken and deposit observed for parasites). The eyes and oral cavity of sampled fish were also scraped (Aloo et al., 2004) Smears were taken from the body surface, fins and gills, spread on a dry clean slide, air dried and fixed with absolute methyl alcohol for 5 minutes. The fixed smears were stained with freshly prepared Giemsa stain for 30-45 minutes after which the film rinsed with tap water and left till dry in air according to Lucky (1977).

2.7. Examination of fish for internal parasites

A cut was made on the ventral side from the anal opening to the lower jaw. Then, two more cuts were made on the lateral side to expose the body cavity with alimentary canal and other internal organs. The surfaces of the visceral organs and body cavities and serous membranes were examined for parasites by using hand lens. Moreover, the alimentary canals were removed and cut into parts (stomach and intestine) in 0.09% physiological saline for parasite recovery under a dissecting microscope. Each part was further carefully slit open to aid the emergence of parasites. Gastrointestinal parasites were further recognized by their wriggling movements on emergence in the normal saline under the microscope Marcogliese et al., (2011).

2.8 .Parasitological processing

The collected helminthes were first relaxed in warm water, and then fixed in 5% formalin and transferred to 70% ethanol. They were processed by the methods previously recorded as they stained with acetic acid alum carmine, destained in 70% acid ethanol (i.e. ethanol with several drops of HCl), dehydrated through a graded ethanol series, clarified in clove oil, and mounted in Canada balsam as permanent preparations, Carleton (1967). The isolated gill worms were stretched between slides and cover slip and fixed by glycerin-alcohol(in ratio 1:4).Then the cover slip was framed with dense Canada balsam and kept in horizontal position, Lucky (1977). The detected crustacean were transferred into petri dish or test-tube containing lactophenol which converse and

clean the parasites then mounted by addition of few drops of Canada balsam on the slide and examined microscopically.

2.8. Identification of parasites:

The identification of the parasites were done according to Yamaguti (1959, 1961 and 1963), also by using the description given by the previous res researchers in this field.

2.9 Data Analysis

The obtained data was analyzed by chi-square test to assess the significant differences in prevalence rates by the aid of SAS software (2004) and differences were considered significant when $p < 0.01$.

3. RESULTS

3.1. Morphology of the detected parasites

3.1.1. Protozoa

The following ciliated protozoa were recorded from the skin, fins and gills from the infected *O.niloticus*:

Trichodina spp. (Davis, 1947)

It was recorded from the skin and gills of *O.niloticus*. The parasite was characterized by a denticulate ring of hollow conical structures with flat lateral projections. The centrifugal projections of denticles were semicircular. The macronucleus was large horseshoe shaped with a round micronucleus. Fig (1), Fig (2), Fig (3)

Chilodonella hexasticha (Kiernik, 1909)

It was identified as heart shaped. The ventral side was concave and ciliated were reduced to two longitudinal belts of cilia to body margins that arranged in parallel rows. The cytostome was distinct on the front part of ventral side with a funnel- shaped cytopharynx and two contractile vacuoles. A single oval to round macronucleus as well as round micronucleus were easily seen. Fig (4)

Myxobolus spp (Ishii, 1915)

The spores were detected in stained smears taken from the skin. The spore was oval to elliptical in shape and provided with two polar capsules which appeared pyriform in shape and equal in size. Fig (5)

Ambiphrya spp (Abu-El-Wafe, 1988)

The investigated organism was vase-shaped body. Long cilia around the cytosome are present in addition to another transverse ciliary zone on the body. The foot (stalk) at the posterior end resembles a disc-like basal. The macronucleus was seen to be in from of irregularly twisted and curved ribbon extending along the entire length of the body. The micronucleus was difficult to be seen while the cytoplasm is densely vacuolated. Fig (7)

Apiosoma spp (Abd-El-Khalek, 1998), (Abu-El-Wafe, 1988)

The body was barrel in shape. The cytosome was surrounded by comparatively short cilia; another transverse ciliary zone was present in the body. Foot

(stalk) at the posterior end is short. The macronucleus was usually globular to round in shape and usually present in the posterior half of the body. The micronucleus was oval to spherical in shape when it be detected but in general it is difficult to been seen while the cytoplasm is densely vacuolated especially the anterior half of the body. Fig (8)

3.1.2. Crustacea

. *Lamproglena monodii*, (Capart, 1944)

The adult female was grayish yellow when collected, the body is cylindrical, maggot like. A pair of distinct egg sacs are present and consisting of a long unistriate chains of grayish eggs. Fig(6)

3.1.3. Monogenea

Macrogyrodactylus spp (Gusse, 1961)

It was found to infect the gills, skin and fins of *O.niloticus*. It is a relatively large monogenea gill parasites. The prohaptor composed of two head lobes each bears a head organ and well developed cephalic unicellular gland. The opisthaptor is well developed and delimited from the body proper by a constriction. There are two large equals anchors. Fig (11)

Gyrodactylus spp (Von Nordmann, 1832)

It is a small monogenea. The body is divided into head region, trunk, peduncle and haptor. The haptor contains a pair of hamuli and provided with two connecting transverse bar. Fig (12)

3.1.4. Acanthocephala

Acanthosentis tilapiae (Baylis, 1948)

The adult has two major body regions. One region, at the anterior end of the body, is a retractable proboscis. The second region, posteriorly, is the trunk, or soma. The trunk is the main body and is generally cylindrical in shape, Sexes are separate. Male and female gonads generally float within the body cavity, and the genital pores of both sexes are at the posterior terminus of the body. The proboscis is covered with hooks, and is attached to the trunk by a neck. The proboscis retracts into a proboscis sheath. Fig (10)

3.1.5. Digenea

Metacercaria of *Clinostomum* spp, (Ukoli, 1966)

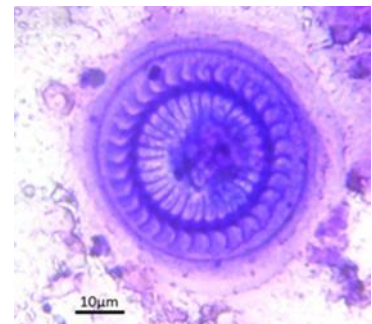
The larva was stout, convex dorsally and concave ventrally. The ventral sucker was large and spherical in shape. The esophagus was lacking and the intestinal caeca were very long extended to the posterior end and provided with lateral pouches behind the ventral sucker. The two testes were strongly digitated, subdivided into small lobes and situated in the posterior part of the middle third of the body. Fig(9).



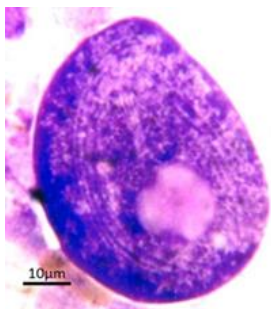
Fig(1)*Trichodina fultoni* x100
Skin and gills of *O.niloticus*
Giemsa stain



Fig(2)*Trichodina californica* x100
Skin and gills of *O.niloticus*
Giemsa stain



Fig(3)*Trichodina truttae* 100 x
Skin and gills of *O.niloticus*
Giemsa stain



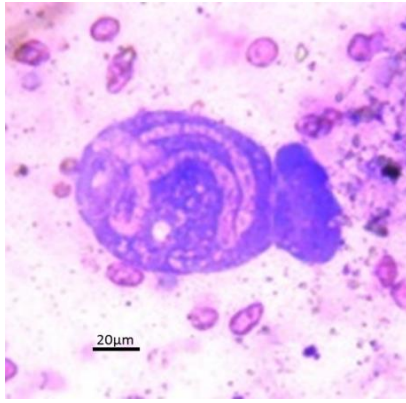
Fig(4)*Chilodonella* spp 100x



Fig(5)*Myxobolous* spp 100 x
Skin and gills of *O.niloticus* Giemsa stain



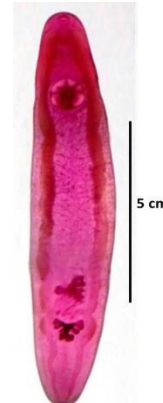
Fig(6)*Lamproglena monodii* 10 x
Gills of *O.niloticus*.



Fig(7)*Ambiphrya* spp. (x40) Skin and gills of *Tilapia* spp. Giemsa stain



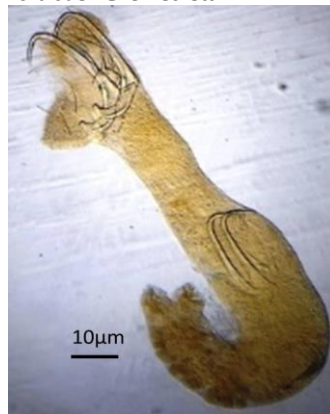
Fig(8)*Apiosoma* spp Skin and gills of *O.niloticus* Giemsa stain



Fig(9)*Clinostomum* spp 10 x



Fig(10)*Acanthosentis tilapiae* Male 10x



Fig(11)*Macrogyrodactylus* spp 10 x



Fig(12) *Gyrodactylus* spp 10 x

3.2. Incidence of the detected parasites

Out of 360 examined fish specimens from *O.niloticus*. were collected ;199 fish were found to be infested with a total infestation rate 64.5 %. Idku Lake recorded high parasitic burden by (72.5%) followed by Edfina by (56.5 %) then Mariout lake with the lowest level of parasitic infestation by 36% (Table 1) & (Fig13).

3.3. Occurrence and prevalence of the revealed parasites: From the examined fish, Ciliates protozoan parasites predominated than other

parasites where it recorded the highest incidence 83.4% followed by Monogenic trematodes, Crustacea and Acanthocephala by 21.6%, 19.09% and 7.53% respectively while the infestation rate with Digenia was the lowest by 2%.

3.4 Prevalence of different external and internal parasites among the examined *O.niloticus*, Table (2), (3), (4), (5) & Fig (14).

3.5 Water Analysis: Table (6), (7) represent a summary of variations in the chemical variables in the different sampling sites

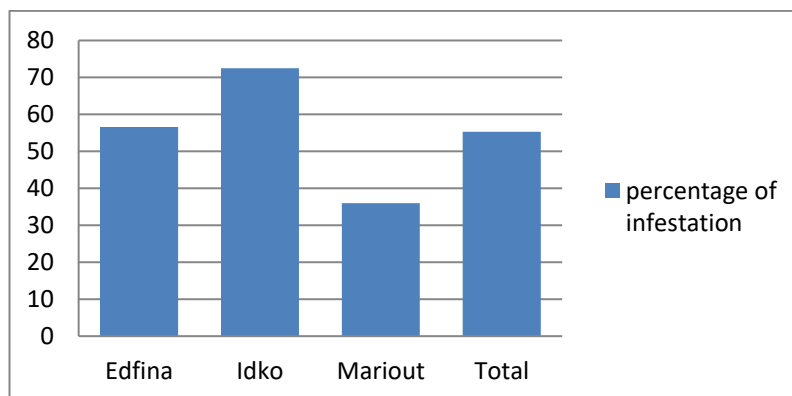


Figure (13): Total incidence of infestation among examined *O.niloticus*.

Table (1) Total number of parasitic infestation among examined fishes .

Site	No. of examined fish	No of infested fish	%
Edfina	120	68	56.5
Idku	120	87	72.5
Mariout	120	44	36
Total	360	199	64.5
Chi-Square	6.58* (P<0.05)		

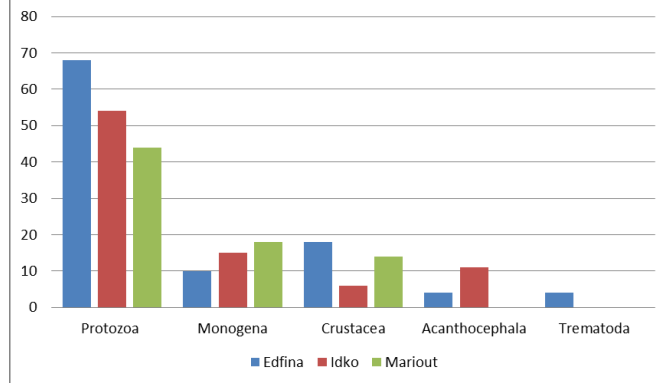


Figure (14): Occurrence of different parasites among examined *O. niloticus*.

Site	Edfina	Idku	Mariout	Chi-Square
Exam. No	120	120	120	
Total infestation	68	87	44	
Protozoa	68	54	44	32.77* (P<0.0001)
%of infest	100	62	100	
Monogenea	10	15	18	0.18 NS (P>0.05)
%of infest	14.7	17.24	40.9	
Crustacea	18	6	14	11.17* (P<0.001)
%of infest	22	6.89	31.8	
Digenea	3	1	0	1.61NS (P>0.05)
%of infest	4.4	1.2	0	
Acanthocephala	4	11	0	1.99NS (P>0.05)
%of infest	5.88	12.04	0	

Table (2) Occurrence of different parasites among examined fishes.

Site	Edfina	Idku	Mariout	Chi-Square
Exam.No	120	120	120	
Trichodina spp	68	23	30	35.84* (P<0.0001)
%of infest	56.6	19.1	25	
Apiosoma spp	-	54	15	69.68* (P<0.0001)
%of infest	-	45	12.5	
Ambiphrya spp	-	14	10	14.87* (P<0.001)
%of infest	-	11.6	8.33	
Chilodonella spp	10	3	-	3.97* (P<0.05)
%of infest	8.3	2.5	-	
Myxobolus spp	8	2	-	3.76NS (P>0.05)
%of infest	6.6	1.6	-	

Table (3): Incidence of the revealed protozoa among examined fishes.

Site	Tot, exam	Monogenea		Crustacea			
		Macrogryodactylus spp		Gyrodactylus spp		Lamproglana monodii	
		Infest. No.	% infest	Infest. No	% infest	Infest. No	% infest
Edfina	120	8	6.66	2	1.66	18	15
Idku	120	7	5.83	8	6.66	6	5
Mariout	120	10	8.33	4	3.33	20	16.66
Chi-Square		0.07NS (P>0.05)		3.76NS (P>0.05)		6.67* (P<0.01)	

Table (4): Incidence of the revealed Monogentic trematoda and Crustacea among examined fishes.

Table (5): Incidence of the revealed Digenitic trematoda and Acanthocephala among examined fishes.

Site	Tot.exam	Digenitic trematoda		Acanthocephala	
		<i>Clinostomum spp</i>		<i>Acanthosentis tilapiae</i>	
		Infest. No.	% infest	Infest. No.	% infest
Edfina	120	3	2.5	4	3.33
Idku	120	1	0.8	11	9.16
Mariout	120	0	0	0	0
Chi-Square		1.02NS (P>0.05)		3.48NS (P>0.05)	

Table (6): Chemical water-quality criteria for Idku Lake, Edfina and Mariout Lake.

Water quality parameter	Permissible limits	Edfina	Idku	Mariout
pH	7-8.5	8.98	7.45	7.29
TSD Total soluble solids mg/L	300	539	2263	5368
Total Alkalinity ,mg CaCO ₃ / L	25-100	40.0	66.5	44
Ph ph Alkalinity ,mg CaCO ₃ / L	-	0.0	6.0	0
Bicarbonate HCO ₃ , mg CaCO ₃ / L	-	40.0	56.5	44
Total Hardness mg CaCO ₃ / L	75-150	217	610	1350
Calcium Hardness mg CaCO ₃ / L	-	132	194	650
COD(organic matter)mg/L	2-3 mg/L	80.00	12.80	96
Ammonia (mg L-1)	0- <0.025	0.42	0.03	0.0054
Nitrite No ₂ (mg L-1)	<0.02	0.26	0.09	<0.005
Nitrate No ₃ (mg L-1)	0.1-4.5	1.23	0.24	0.0063
Phosphate Po ₄ (mg L-1)	0.01-3	0.08	0.03	<0.005
Calcium mg/L	75	57.906	77.755	250.5
Magnesium mg/L	30	20.655	101.088	170.1
Sulfate So ₄ mg/L	200	230.22	514.50	803.4
Sulfides H ₂ S mg/L	0.002	<LOD	<LOD	2

Table (7) Average heavy metal concentrations in Idku Lake, Edfina and Mariout Lake (mg/l-1)

Parameter	Permissible limits	Edfina	Idko	Mariout
Fe µgl-1	< 1000	0.014	0.042	0.292
Mn µgl-1	50 - 500	ND	ND	0.25
Zn µgl-1	50	ND	ND	0.146
Cu µgl-1	30	0.0020	0.0018	0.00413
Pb µgl-1	50	ND	ND	-
Cd µgl-1	10 - 30	ND	ND	-
Ni µgl-1	10-30	ND	ND	-

ND: Not detectable

4. DISUSSION

The present study was conducted to compare between effects of water pollution on the parasitic community of *Oreochromis niloticus* in Edfina (Natural Nile resources), Lake Idku and Lake Mariout. This work revealed a total infestation rate of 64.5% that 199 fish were found to be infected with different parasites species out of 360 examined fishes this results come in agreement with that reported by Abdel-Mawla and El-El-Ekiaby (2012) who mentioned that the total infestation rate (64%) with different parasites among examined

freshwater fish in of Suez canal water at Ismailia province.

Concerning fish species, *O. niloticus* was chosen to study the impact of pollution in three locations due to its wide range of food selection and its tolerance to chemicals and physical pollutant factors. (Ramadan et al, 2014)

The prevalence of Parasitic Protozoa in this study was (83.4 %) in total examined *O. niloticus*. This results is found nearly similar results recorded with El-Shanat (2007) who reported an infestation rate of (72.5%) and higher than those obtained by El-Seify et al., (2011) and Noor El dean et al

(2014) who reported an infestation rate of (55.5%) and (22.5%) respectively. Dealing with *Acanthocephala* which showed a total infestation rate of (7.53%). This result agreed with Abdel-Mawla and El-El-Ekiaby (2012) who reported *Acanthocephala* with a total infestation rate (7%) and nearly similar with the results obtained by (Youssef and Derwa, 2005 and El-Lamie, 2007) in which the percentage were (5.6% and 5%) respectively. These results disagree with results obtained with Shahat et al, (2011) who reported *Acanthocephala* with a total infestation rate (32.5%) from *O. niloticus* fishes collected from Assuit province.

The examination of *Oreochromis niloticus* revealed that they harbored the digenetic trematode metacercaria, *Clinostomium* spp in the gills. with a total infestation rate (2%) these results nearly similar with the results obtained by EL-Shahway et al (2017) who studied the endoparasites of 72 *O. niloticus* fishes in southern region of Egypt and reported *Clinostomum tilapiae* with a prevalence (4.6%) and disagree with these recorded with Bichi and Ibrahim (2009) who found prevalence rate of 21.2% due to *Clinostomum* spp. in *Tilapia* spp.

The present investigation revealed that Monogenetic trematodes recorded a total infestation of (21.6%). These results nearly similar with result obtained with El-Shanat (2007) who reported an infestation rate of (17.7%) These results disagree with those obtained by El-Seify et al., (2011) and Noor El dean et al (2014) who reported an infestation rate of (33.6%) and (11.5%) respectively with *Monogenetic trematodes*. which may be due to different of sample collection and changes in water quality

The prevalence of Parasitic crustaceans in this study was (19.09%) in total examined *O. niloticus*. This result is nearly similar those obtained by Abd El-Khalek (1998) who recorded that the prevalence was (24.73%), and higher than those obtained by El-Shanat (2007) and Noor El dean et al (2014) who recorded an infestation rate (9%) and (3%) respectively.

Regarding water analysis, although both Edfina and Mariout show elevation in water quality parameters than permissible limits, but Mariout consider being heavily polluted than Edfina; which is polluted. The increase in Nitrite content at Edfina may be due to the increase in microbial activity and nitrification process. While, the increase in Ammonia may be pointed to the industrial effluents and agricultural drainage water which discharged directly into Edfina and this agrees with the finding

of (Svobodova et al., 1993 and Anzfc and Armcanz, 2000).

The results showed that there were inverse relationship between parasitic infestation and water pollution. These results agreed with Valtonen et al (1997) and Dzik (2003) who mentioned that water pollution effect directly on monoxenous parasites (gill parasites) which developed more intensity after the adaptation to the surrounding and indirectly on heteroxenous parasites (intestinal parasites) by decrease the abundance of the intermediate host therefore decrease its abundance and also with Nachev and Sures (2009) and Chapman et al., (2015) reported higher parasite diversity at less polluted sampling sites, whereas the composition of the parasite fauna and the abundance of some parasites showed a clear relationship with the pollution gradient. These results disagree with Khan (2004) and Abdel-Mawla and El-Ekiaby (2012) who mentholated that the parasitic infestation increased in the highly polluted water and Shafi et al (2015) who stated that changes in the water quality under anthropogenic pollution have not resulted in increase in the parasitic load. This may be due to different in sample collection, fish species and water quality in different localities.

Concerning Ciliated protozoan Edfina recorded an elevation in nitrite, nitrates and ammonia which increase the abundance of protozoal infestation especially *Trichodia* spp than Idku where these parameters were within the permissible limits and Mariout where these parameters were completely absent. These results agreed with Ocute and Palm (2005) and Dzikowski et al (2003) who reported a high prevalence of *Trichodina* spp in water polluted with nitrite, nitrates and phosphate.

Dealing with the incidence of *Acanthocephala*, although the difference between Edfina and Idku was not significant but Idku recorded a high infestation rather Edfina due to its elevation in PH than the permissible limits. These results agreed with Marcogliese and Cone (1996) who mentioned that water with increased PH held no snails which precluded infection with digeneans while gammarids-intermediate hosts of acanthocephalan developed more intensity.

The reason for high prevalence of acanthocephala infection as compared to the other kind of parasitic infections might be due to the low host specificity of the adult stages of these parasites which makes them capable of infecting different fish genera and species. It may also be because of the availability of the different host required for the completion of the life cycle of these parasites Yanong (2002).

Theses difference in the rates between the different localities may be attributed to the difference in the environmental condition, fish species and the difference in the degree of water pollution as well as numbers of examined samples.

5. CONCLUSIONS

The effects of pollution may be important for parasite survival and establishment. Toxic pollution reduces the diversity of heteroxenous parasites, whereas parasites with direct life cycle (monoxenous) are less affected. The results of the present study suggest that the changes in the structure of a parasitic community reflect differences in the composition of the aquatic species, so that this study can support the hypothesis that parasites communities are a good indicators of environmental stress. On the other hand may enable parasitologists to consider and treat parasites not only as creatures that threaten the health of their hosts but also as responsive organisms with applied bioindication value in an environmental sense.

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