

Histopathological, Haematological and Biochemical Indices of *Clarias gariepinus* (Burchell, 1822) Parasitized by Endoparasitic Fauna in Fish Farm of the Northeastern Egypt

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How to cite

Radwan, M., Shehata, S., Abdelhadi, Y., Mohammed, R., Mohamed, M., Magdy, M. (2021). Histopathological, Haematological and Biochemical Indices of *Clarias gariepinus* (Burchell, 1822) Parasitized by Endoparasitic Fauna in Fish Farm of the Northeastern Egypt. *Turkish Journal of Fisheries and Aquatic Sciences*, 21, 465-478. http://doi.org/10.4194/1303-2712-v21_9_05

Article History

Received 23 January 2021

Accepted 30 May 2021

First Online 03 June 2021

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Keywords

Histopathology

Blood indices

Clarias gariepinus

Endoparasitic fauna

Fish farm

Abstract

Knowledge of the endemic fauna of parasites in fish are of great importance, parasites could synergistically act as stressors to health of their hosts. The objectives of this study were to evaluate prevalence and effect of parasites on hematological, biochemical indices, injury of tissues and length weight relationship of *Clarias, gariepinus*. Water quality variables were measured and fishes were collected each seasons for examination most common parasites in infected fishes at Abbasa Fish Farm, Egypt. Results indicated that high summer water temperature was strongly associated with parasites infection. The hematological and biochemical analysis showed significant reduction in red blood cells (RBCs) count, hemoglobin (Hb) value, packed cell volume (PCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), total protein, Albumin, Globulin and A/G ratio, while total white blood cells (WBCs) count, mean corpuscular volume (MCV), aspartate aminotransferase activity (ASAT), alanine aminotransferase activity (ALAT), urea, creatinine, uric acid and glucose were significantly increased in infested catfish. On other hands, histopathological examination of infected fish indicated, organs most affected by infection of parasites. Overall the tendencies observed in data showed the parasites have a strong effect on host fish and drifts observed for all variables showed a strong seasonal decoration.

Introduction

Aquaculture has been an expert in Egypt for millennia, but modern approaches have only recently been adopted to maximize its output. Today, aquaculture production in Egypt is the largest in Africa, with approximately one million tonnes per annum (Shaalán *et al.*, 2018). Diseases caused by parasites are widespread and cause fish losses in the intensively stocked pond and aquarium (Koyuncu & Toksen, 2010). Fish parasites constitute the leading cause of economic losses in aquaculture, including various types of

pathogens, causing deformity, weight loss, mortality, etc. (Eissa, 2002).

Parasites can also affect the energy metabolism of their hosts, in most host-parasite systems; parasitism increases the host's metabolic rate (Devevey *et al.*, 2008).

Hematological parameters constitute an important tool that reveals the health state of fish. Parasitism may induce lowered growth and hematological alteration; this alteration may affect the natural resistance of fish to parasites. On the other, hands certain blood parameters serve as a reliable indicator as many

parasites can live, sometimes causing damage to their hosts (Martins *et al.*, 2004).

Sabri *et al.* (2009) investigated the impact of the parasitic infestation with parasitic on the hematological parameters of the catfish, *C. gariepinus*. Results revealed that parasitic causes physiological dysfunction on the infested fish by showing several alterations in hematological parameters that may cause anemia by reducing erythrocytes (RBCs) count, hemoglobin content, and packed cell volume. Also, Bahaa (2012) recoded that hematological results showed anemia in fish infested by encysted metacercariae than non-infested ones. Hemoglobin content decrease, plasma glucose concentration mainly increased, decreased total plasma proteins, increased blood plasma enzyme activities (AST, ALT). However, the hematological manifestation of the infected fishes showed a marked decrease in the content of hemoglobin concentration (Hb), packed cell volume (PCV) and red blood cells. In general, the infected fishes had a higher range of white blood cell (WBC) than the uninfected one Nnabuchi *et al.* (2015).

With a broad range of causes, fish diseases and histopathology can be used as environmental stress indicators since they provide a definite biological end point of histological exposure. It is a mechanism that can indicate fish health by determining early injury to cells and can therefore be considered an important tool to assess the effect of parasites on fish tissue (Fartade & Fartade, 2016). Moreover, histopathological biomarkers can be sensitive indicators of subcellular stress in organisms exposed over short and long periods to a range of pollutants (Adams *et al.*, 2000). These biomarkers embody tissue lesions arising from previous or current exposure of the organism to one or more toxins (Velkova- Jordanoska & Kostoski, 2005).

Fish diseases and histopathology, with a broad range of causes can be used as indicators of

environmental stress, since they provide a definite biological end point of histological exposure, it is a mechanism which can provide an indication of fish health by determining early injury to cells and can therefore be considered an important tool to determine the effect of parasites on fish tissue (Fartade & Fartade, 2016). Moreover, histopathological biomarkers can be sensitive indicators of subcellular stress in organisms exposed over short and long periods to a range of pollutants (Adams *et al.*, 2000).

Clarias gariepinus (Burchell, 1822) is of great importance for livelihoods representing an income source in many parts of Africa. Parasites have the potential to drastically impact the aquaculture of this fish species (Walakira *et al.*, 2014) and therefore, the health of this fish is of significant concern. Many endoparasitic helminths have been reported to infect *C. gariepinus* (Kuchta *et al.*, 2018) suggesting that the fish plays an important role in life cycles of many parasites in freshwater ecosystems, parasites, particularly helminths *C. gariepinus* hosts the highest richness of adult helminth species in Africa (Khalil & Polling, 1997) hence, it is a suitable model to study the effect of parasites on Hematological, biochemical and histopathological changes in the infected fishes, moreover, threw light on the changes in length-weight relationship and condition factor of infected fishes.

Material and Methods

Study Region

Abbasia Fish Farm, one of the most productive Egyptian fish farms, is located in EL-Sharkia Governorate between latitudes 30° 32' North and latitudes 31° 44' East. The fish farm is about 890.31 hectare. Fish farm is supplied with water from Ismailia Canal (Figure 1).



Figure 1. Image of google earth map showing the location of the study area

Water Quality Parameters

Water samples were collected from each pond for different water physicochemical parameters; temperature, dissolved oxygen, pH, alkalinity, total ammonia (NH₃), hydrogen sulphid (H₂S) and oxygen consumed and dissolved oxygen. Water samples were analyzed according to methods described by Boyd and Tucker (1992).

Collection of Fish Samples

A total of 694 *Clarias gariepinus* cultivated fishes to cover two categories; firstly, sized groups, small sized group (25-39.9 Cm) while, large sized group; being 40 - 55 Cm, the second category was according to sex of *C. gariepinus* were collected seasonally from October 2015 to September 2017. After collection, fishes were transferred alive to El-Abbassa laboratory, then, were macroscopically examined for parasites.

Clinical Examination

After collection, the total mean length and weight of fish species were recorded and then clinical examination was done on the live fish or freshly dead ones. Fish specimens were grossly examined for determination of any clinical abnormalities and any external parasites or visible cysts according to the methods reported by EL-Shahawy *et al.* (2017).

Examination of Fish for Parasites

Fishes were sacrificed by cervical dislocation prior to dissection. 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 the alimentary canal and other internal organs. The surfaces of the visceral organs and body cavities and serous membranes were examined for parasites using a hand lens. Moreover, the alimentary canals were removed and catted 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, 2011).

The collected cestode and trematode were first relaxed in hot water and then fixed in 5% formalin and transferred to 70% ethanol after one week (Oros *et al.*, 2010). For study under the light microscope, specimens 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. All recovered helminths were identified by using the texts of (Yamaguti, 1961 & Cheng, 1973).

Hematological and Biochemical Analysis of the Fish Samples

Hematological examination, total RBCs count and WBCs count were determined by using an improved Neubair hemocytometer (Hesser, 1960) and the packed cell volume (PCV) was determined by using microhematocrit capillary tube (Wintrobe, 1967). Hemoglobin content in blood was determined by using Diamond diagnostic haemoglobin kit (Wintrobe, 1965). The other blood indices such as mean corpuscular volume (MCV) mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). While that of enzymes; aspartate amino transaminase (AST), alanine aminotransferase (ALT), glucose and total protein were preserved in bottles without anticoagulant. Were also calculated by using standard formula according to Dacie and Lewis (1975).

Histopathological Study

Fish organs gills, muscle and liver, experienced fixation process for 24 h using 10% formalin solution. The next steps were dehydrating, clearing, paraffin infiltration, blocking, cutting/sectioning, staining, and mounting. A light microscope was used to analyze the samples. The histopathology analysis was based on the book of Genten *et al.* (2009).

Length-weight Relationship and Condition Factor

After collection fishes, standard length of each fish was measured to the nearest millimeter. The body weight was also determined to the nearest 0.1 gram and recorded. The length-weight relationship was determined using power equation or its logarithmic modification according to the followings method as follows:

$$\text{Log } W = \text{Log } a \pm b \text{ Log } L \text{ (Lagler, 1956).}$$

Where:

W= Weight of the fish in gram.

L= Standard length in centimeter.

a and b = Constants, whose values are estimated by the least square method (Lagler, 1956).

By grouping the fish into 10 mm length groups, the empirical and calculated weights were determined.

The length-weight relationship can be expressed in terms of condition factor (K), which measures the well-being of fish. Such factor was calculated from the equation proposed by Hile (1936) as follows:

$$K = 100 W/L^3$$

Where:

W= Fish weight in gram.

L= Fish length in centimeter.

The relative condition factor (Kn) was determined by the following formula Hile (1936):

$$Kn = W/w$$

Where:

W= Fish weight in gram.

w= Calculated weight estimated from the length-weight relationship.

Data Analysis

Prevalence of parasites were estimated through the following formula (Margolis *et al.*, 1982)

Prevalence = number of individuals of a host species infected with a particular parasite species ÷ number of hosts examined × 100.

Statistical analysis. Data were analyzed by using multivariate ANOVA analysis at (P<0.05) using the SPSS (13.0) statistical program.

Results

Physico-chemical Characters

Table 1 revealed that, maximum average value of surface water temperature was recorded during summer (28.14±1.45 °C) and the lowest (16.43±1.12 °C) during winter, concerning hydrogen ion concentration (pH), the highest average value was recorded during winter (8.77±0.47), and reached its lowest value (7.23±0.30) during spring. Regarding the minimum average value of turbidity was recorded during spring (44.17±7.04 % and the maximum value (75.86±4.69 %) was recorded during winter. On the other hand, highest average value of total ammonia (1.27±0.42 mg/L) was recorded during summer and the lowest (0.74±0.07 mg/L) was recorded during spring. In contrast, the maximum average value of total alkalinity was recorded during summer (242.67±33.55 mg/L), and reached its minimum value (176.00±20.30 mg/L) during spring. Moreover, the minimum average of hydrogen sulfide concentration was recorded during spring (0.72±0.16 mg/L), and reached its maximal value (1.02±0.13 mg / L) during winter. Additionally, the highest value of oxygen consumed was recorded during winter (8.12±0.39 mg/L) and reached its minimum value (6.01±0.22 mg/L) during spring. On the other hand, the maximum values of

dissolved oxygen were recorded during winter (8.94±0.75 mg/L) and autumn (8.36±0.65 mg/L) and the minimum values were detected during spring (6.85±0.45 mg/L) and summer (6.42±0.69 mg/L).

Clinical Signs and Post-mortem Lesions in the Infected Fish

In general, clinical signs in the infected fish, *Clarias gariepinus*, revealed some clinical change on the external body surface. Some infected *C. gariepinus*, however, showed ulcer-like structure at the skin surface, increasing secretion of mucous, whitish cysts at muscles and liver were observed (Figure 2).

Seasonal Dynamics of Parasitic Infection in *Clarias gariepinus*

Results showed that the highest parasitic infection in *C. gariepinus* was recorded during summer (87.82 %); it decreased gradually during spring (79.03 %), through autumn (75.50 %), and reached its lowest value during winter (55.81%) (Table 2&3).

Seasonal Prevalence of Different Parasites in *Clarias gariepinus* According to Sex and Sized Groups

Table 4 revealed that the prevalence of protozoan in *C. gariepinus* was recorded during autumn (11.26%) and the minimum value (6.99 %) was observed during spring. During summer and winter, however, protozoan prevalence was nearly similar; being 8.12 and 8.14 respectively. The maximal value of parasitic trematodes was recorded during summer (30.26 %); it decreased gradually during spring (29.57 %) through autumn (27.81 %) and reached its minimal value during winter (22.09 %).

Concerning nematodes, the highest value was recorded (20.66 %) during summer and the lowest (15.12 %) during winter. During spring and autumn, however, nematodes prevalence was nearly similar; being 17.20 % and 18.54 % respectively. The highest value of cestoda prevalence was recorded during summer (28.78 %); it decreased gradually during spring (25.27 %) through autumn (17.88 %) and reached its lowest value (10.47 %) during winter. The total value of seasonal dynamics in the examined *C. gariepinus*, along the year round was detected highest prevalence of

Table 1. Seasonal variations of physico-chemical factors in Abbassa Fish Farm waters.

Seasons	Spring	Summer	Autumn	Winter	Annual average
Temperature (°C)	23.24±1.35	28.14±1.45	22.49±1.29	16.43±1.12	22.57 ±1.30
pH	7.23±0.30	7.57±0.52	8.24±0.35	8.77±0.47	7.95 ±0.41
Turbidity (%)	44.17±7.04	56.52±4.13	73.99±5.57	75.86±4.69	62.63 ± 5.36
Total ammonia {NH ₃ (mg/L)}	0.74±0.07	1.27±0.42	0.86±0.05	0.85±0.07	0.93 ± 0.15
Total alkalinity (mg/L)	176.0±20.30	242.67±33.55	200.00±26.00	222.0±21.93	210.17±25.44
H ₂ S (mg/L)	0.72±0.16	0.88±0.21	0.96±0.16	1.02±0.13	0.90 ± 0.16
Oxygen consumed (mg/L)	6.01±0.22	6.57±0.15	7.77±0.57	8.12±0.39	7.12 ± 0.33
Dissolved Oxygen (mg/L)	6.85±0.45	6.42±0.69	8.36±0.63	8.94±0.75	7.64 ± 0.63

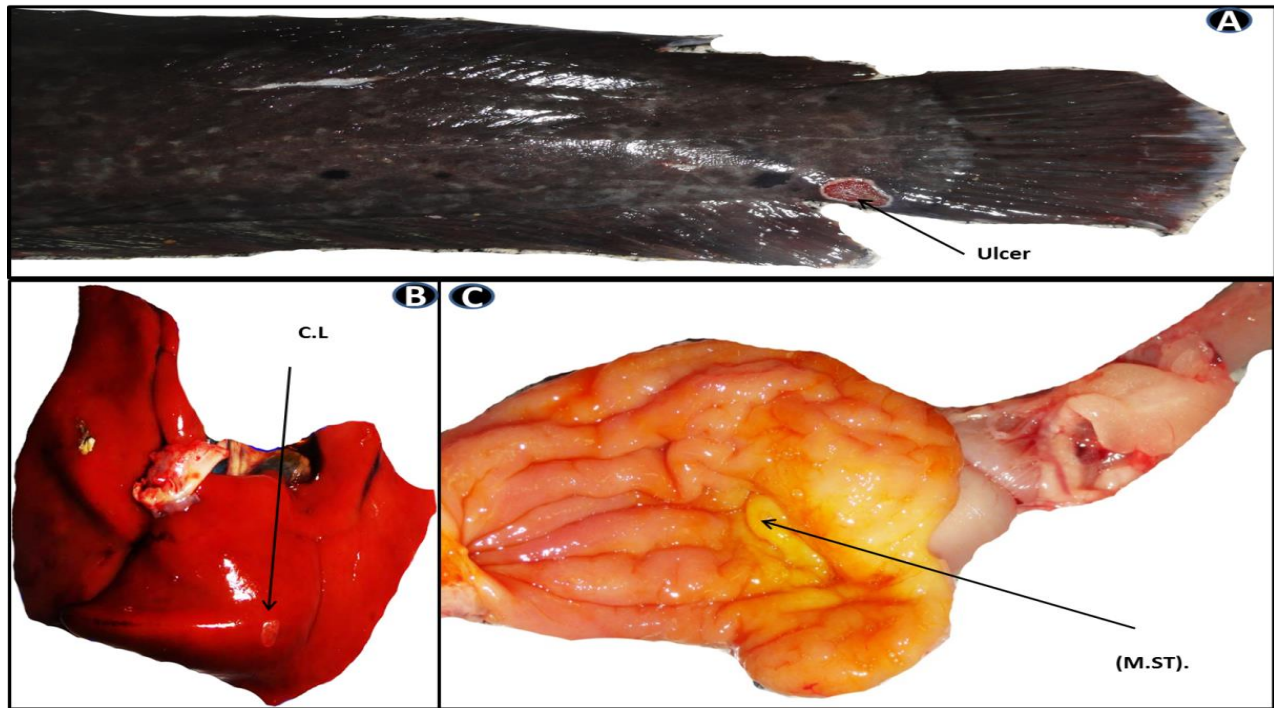


Figure 2. Image of *Clarias gariepinus*, showing ulcer like patches at the skin (A), whitish cyst at the liver (C.L) (B) and increasing mucous in stomach (M.ST) (C).

Table 2. Seasons varitione of parasites among the examined fish collected from Abbasa Fish Farm.

Season	No. of examined fish	No. of infected fish	Percentage (%)
Spring	186	147	79.03
Summer	271	238	87.82
Autumn	151	114	75.50
Winter	86	48	55.81
Annual average	173.5	136.8	74.54

Table 3. Analysis of variance (ANOVA) performed on the percentage of infection of *C. gariepinus*, collected from Abbasa Fish Farm, according to sex and sized groups.

Source of variance	F-value
Season	14.30**
Size	193.84 **
Sex	24.62 *
Season * Size	3.03 NS
Season * Sex	13.01 **
Size * Sex	16.38 **

Note * = Significant at $P < 0.05$. ** = highly significant at $P < 0.01$. N.S. = non-significant.

Table 4. Seasonal dynamics of parasitic infection in *C. gariepinus*, collected from Abbasa Fish Farm.

Family	Seasons	Spring	Summer	Autumn	Winter	Totals
Protozoan	No. of examined fish	186	271	151	86	694
	No. of infected fish	13	22	17	7	59
	Percentage of infection(%)	6.99	8.12	11.26	8.14	8.50
Trematodes	No. of infected fish	55	82	42	19	198
	Percentage of infection(%)	29.57	30.26	27.81	22.09	28.53
Nematoda	No. of infected fish	32	56	28	13	129
	Percentage of infection(%)	17.20	20.66	18.54	15.12	18.59
Cestoda	No. of infected fish	47	78	27	9	161
	Percentage of infection(%)	25.27	28.78	17.88	10.47	23.20

trematodes parasitic (28.53%) and the lowest (8.50 %) was recorded in protozoans (Figure 3). Moreover, seasonal dynamics in this fish can be ordered as the following: trematodes>cestodes>nematoda>protozoan.

Parasitic Infection Effects on Haematological and Biochemical Indices

Results (Tables 5-7 and Figure 3) revealed that red blood cell count (RBCs), hemoglobin content and packed cell volume (PCV) decreased in the infected *C. gariepinus*, compared with the uninfected ones. Moreover, the average values of white blood cell counts (WBCs) mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in this fish increased with the increasing infection and decreasing size of this fish. Also, it was higher in females than in males. However, aspartate aminotransferase activity (ASAT), alanine aminotransferase activity (ALAT), Urea, Creatinine, Uric

acid and Glucose were increased in the infected *C. gariepinus* uninfected one in both sized groups. Also, data declared that the total protein, Albumin, Globulin and A/G ratio in the serum of *C. gariepinus*, was decreased with the infection in both sized groups.

Effect of Parasitic Infection on Length-weight Relationship and Condition Factors

Data (Table 8) exhibited a total length of *C. gariepinus*, varied from 25 to 55 Cm, while the total weight ranged between 154 and 1058 g. The weight of the fish increased with the increasing length of the fish. The following equation expresses the length-weight relationship of this fish:

$$\text{Log W} = -1.15 + 2.40 \log L$$

Consequently, the values of "a" and "b" were: -1.15 and 2.40, respectively. The equation mentioned

Table 5. Effect of parasitic infections on haematological indices of *C. gariepinus*, collected from Abbasa Fish Farm, according to sex and sized groups.

Sizes Indices	Sex	Small fish		Large fish	
		Uninfected	Infected	Uninfected	Infected
RBCs ($\times 10^6$ cell / mm^3)	Males	2.53 \pm 0.07	1.91 \pm 0.16**	2.74 \pm 0.14	2.16 \pm 0.15*
	Females	3.80 \pm 0.29	2.47 \pm 0.48**	4.47 \pm 0.56	3.27 \pm 0.24*
WBCs ($\times 10^3$ cell / mm^3)	Males	19.66 \pm 1.74	22.07 \pm 0.24**	17.87 \pm 0.36	18.33 \pm 1.41*
	Females	16.31 \pm 0.72	19.51 \pm 0.97**	15.29 \pm 0.29	17.84 \pm 0.51**
Hb (g/dl)	Males	12.86 \pm 1.41	10.47 \pm 1.20**	14.87 \pm 1.33	11.18 \pm 1.10**
	Females	11.70 \pm 1.52	8.16 \pm 0.35**	13.86 \pm 0.47	10.07 \pm 0.70**
PCV (%)	Males	30.02 \pm 0.81	24.39 \pm 2.13**	34.13 \pm 0.92	28.53 \pm 1.15**
	Females	35.16 \pm 2.77	27.88 \pm 1.17**	37.62 \pm 1.75	30.31 \pm 0.62**
MCV (fl)	Males	118.97 \pm 6.37	129.17 \pm 17.60**	124.68 \pm 6.28	133.10 \pm 14.83**
	Females	93.00 \pm 10.52	116.34 \pm 16.85**	85.08 \pm 7.42	93.05 \pm 6.55**
MCH (pg)	Males	50.79 \pm 4.12	54.75 \pm 1.91	54.17 \pm 3.57	52.03 \pm 6.34*
	Females	30.89 \pm 4.50	34.50 \pm 7.26**	31.50 \pm 4.09	31.09 \pm 4.38 ^{NS}
MCHC (%)	Males	42.96 \pm 5.43	43.40 \pm 7.49*	43.65 \pm 4.58	39.24 \pm 4.04*
	Females	33.15 \pm 1.95	29.35 \pm 2.16**	36.91 \pm 1.90	33.28 \pm 2.80**

Note * = Significant at $P < 0.05$; ** = highly significant at $P < 0.01$; N.S. = non-significant.

Table 6. Effect of parasitic infections on liver and kidney indices of *C. gariepinus*, collected from Abbasa Fish Farm, according to sex and sized groups.

Sizes Indices	Sex	Small Fish		Large Fish	
		Uninfected	Infected	Uninfected	Infected
ASAT (U/ml)	Males	112.48 \pm 2.49	127.46 \pm 4.53*	110.22 \pm 3.18	118.26 \pm 2.62**
	Females	91.93 \pm 7.33	111.02 \pm 10.62**	90.23 \pm 1.70	98.61 \pm 2.02*
ALAT (U/ml)	Males	39.77 \pm 1.39	52.36 \pm 7.84**	40.92 \pm 3.39	44.02 \pm 2.09*
	Females	36.97 \pm 2.70	71.36 \pm 2.68***	37.41 \pm 1.32	60.06 \pm 6.37**
Urea (mg/dl)	Males	9.20 \pm 0.58	9.63 \pm 0.55 ^{NS}	10.77 \pm 0.48	11.04 \pm 0.52*
	Females	9.17 \pm 1.22	9.40 \pm 0.59*	8.97 \pm 0.30	9.95 \pm 0.46*
Creatinine (mg/dl)	Males	0.79 \pm 0.04	0.96 \pm 0.10*	0.84 \pm 0.06	0.89 \pm 0.08 ^{NS}
	Females	0.75 \pm 0.05	1.01 \pm 0.09*	0.82 \pm 0.03	0.92 \pm 0.02 ^{NS}
Uric acid (mg/dl)	Males	1.92 \pm 0.14	2.21 \pm 0.32*	1.88 \pm 0.10	1.93 \pm 0.29 ^{NS}
	Females	1.90 \pm 0.16	2.12 \pm 0.24*	1.86 \pm 0.07	1.94 \pm 0.27*

Note * = Significant at $P < 0.05$; ** = highly significant at $P < 0.01$; N.S. = non-significant.

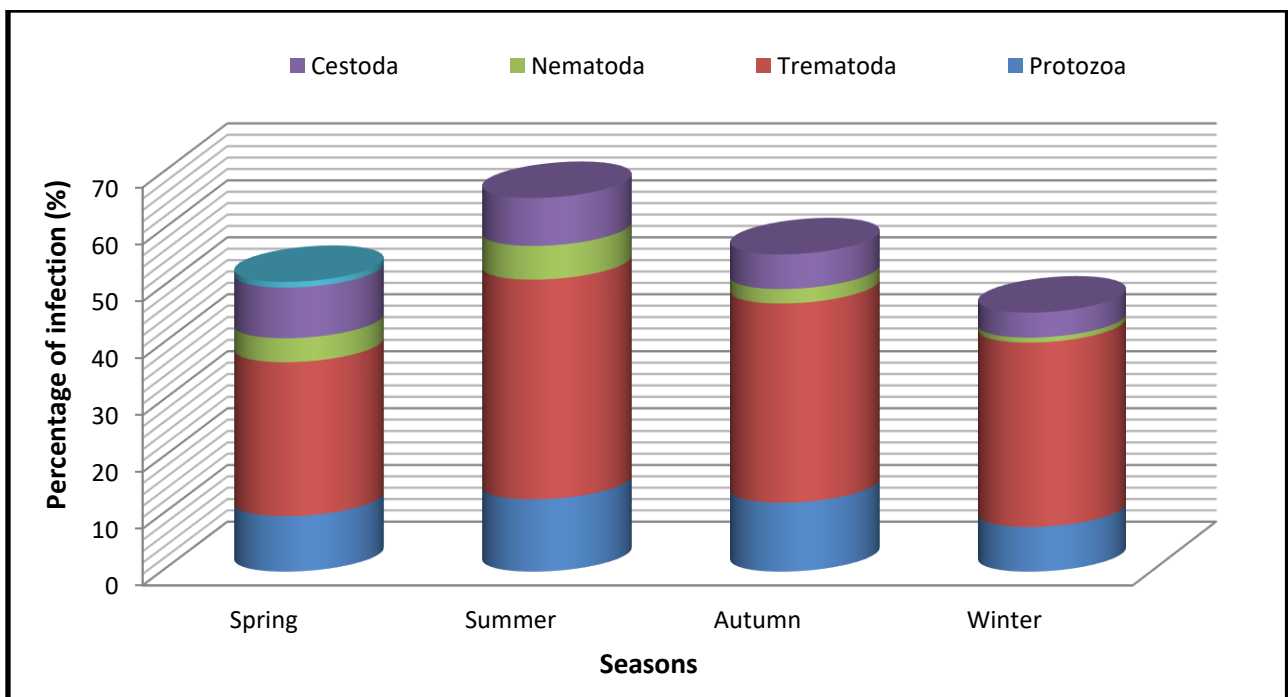
Table 7. Effect of parasitic infections on glucose, total proteins, albumin, globulin and A/G ratio in the serum of *C. gariepinus*, collected from Abbasa Fish Farm, according to sex and sized groups.

Indices	Sex	Small fishes		Large fishes	
		Uninfected	Infected	Uninfected	Infected
Glucose (mg/dl)	Males	68.93±1.83	84.69±3.74***	69.26±0.99	81.13±2.16**
	Females	76.96±4.38	91.64±2.68***	75.76±2.75	82.62±3.42**
Total protein (g/dl)	Males	3.54±0.51	2.49±0.18**	3.66±0.10	2.83±0.27*
	Females	3.15±0.16	2.83±0.13*	3.98±0.11	3.07±0.10*
Albumin (g/dl)	Males	3.47±0.58	2.24±0.33**	4.02±0.15	2.86±0.20**
	Females	3.18±0.39	2.85±0.21*	3.18±0.14	2.99±0.09*
Globulin (g/dl)	Males	2.89±0.23	2.39±0.36*	3.36±0.31	2.43±0.27 ^{Ns}
	Females	2.67±0.54	2.29±0.27*	2.83±0.10	2.32±0.10**
A/G ratio	Males	1.19±0.13	0.94±0.10*	1.21±0.17	1.19±0.12 ^{Ns}
	Females	1.24±0.31	1.25±0.06 ^{Ns}	1.12±0.05	1.29±0.06*

Note * = Significant at P<0.05; ** = highly significant at P<0.01; N.S. = non-significant.

Table 8. Length-weight relationship and condition factors of infected *C. gariepinus*, collected from Abbasa Fish Farm

Length Size class (Cm)	No. of un-infected fish	Observed weight	Calculated weight	Condition factors	
				K	Kn
25 - 27.9	52	182.74±18.58	183.93±14.55	1.01±0.05	0.99±0.05
28 - 30.9	61	240.65±17.48	244.19±18.64	0.93±0.09	0.99±0.08
31 - 33.9	57	309.42±27.50	±19.53 307.39	0.90±0.05	1.01±0.05
34 - 36.9	92	373.76±38.43	374.10±20.16	0.85±0.06	1.00±0.07
37 - 39.9	86	473.76±35.43	459.26±26.40	0.83±0.05	1.03±0.06
40 - 42.9	49	555.75±27.08	27.75 547.82±	0.78±0.03	1.02±0.04
43 - 45.9	39	651.25±33.29	±30.02 649.74	0.74±0.02	1.00±0.02
46 - 48.9	41	±23.04 748.68	±28.52 758.48	0.70±0.03	0.99±0.03
49 - 51.9	36	28.60 869.13±	±37.21 877.32	0.68±0.02	0.99±0.03
52 - 55	34	1002.08±35.28	1013.18±37.97	0.66±0.02	0.99±0.02
Total	547	540.72±28.47	541.54±26.07	0.81±0.04	1.00±0.04

**Figure 3.** Seasonal dynamics of parasitic infection in *C. gariepinus*, collected from Abbasa Fish Farm.

above observed that the “b” value (2.40) was decreased than the ideal one, indicating a tendency towards slightly negative allometric growth. The correlation coefficient “r²” was 0.98, which is statistically highly significant.

Effect of Parasitic Infection on the Histopathological Changes in *C. gariepinus*

Histopathological examination of muscles in the infected *C. gariepinus* muscles showed multiple parasitic cysts of different types (encysted metacercaria) among muscle fibers, surrounded mainly by adipocytes. The viable cyst is formed from scolex, internal structure, cuticle, and cyst membrane. The degenerated cyst revealed distorted parasitic elements, parts of the cyst membrane (cyst wall), and reactive fibrous capsule. Other cysts showed complete degeneration of the parasitic structures with replacement of the tissue by a reactive inflammatory process of chronic nature, including lymphocytes, histocytes, fibroblast and melanomacrophage cholesterol cleft among the parasitic walls of some cysts. Focal interstitial fibrosis and adipocyte cysts were also recorded. While some muscle fibers showed normal histological structures and hyaline degeneration (Figure 4).

Regarding infected *C. gariepinus*, the liver showed viable parasitic cysts among hepatic parenchyma; they showed a comparatively thick fibrous capsule. Portal aggregation of melanomacrophages, fatty and hydropic degeneration were also detected. Perportal fibrosis with complete disappearance of pancreatic acini and aggregation of melanomacrophages were also observed. Moreover, parts of the liver showed focally ruptured hepatocytes (Figure 4).

Identification of Parasites Isolated from the Examined Fishes

The identified parasites were Protozoa (*Henneguya branchialis*), Metacercaria of digenetic trematodes (*Diplostomum tilapiae*, *Cyanodiplostomum azimi*, *Haplorichis sp*, *Heterphyes sp*, *Pygidiopsis genata*, *Prohemostomum vivax*, *Orientocreadium batrachoides*, Cestoda (*Polyonchobothrium clarias*), Nematoda (*Procamallanus laevionchus* and *Paracamallanus cyathopharynx*) (Figures 5-7).

Discussion

The physico-chemical parameters of a body of water is very important to the productivity, growth and survival of the aquatic organisms that are living in the water and thus play an important role in the biology and physiology of the fishes which are part of the organisms living in water (Owhonda *et al.*, 2007). In the present study, water quality parameters fluctuated throughout the year

Temperature is one of the most important factors which affect the living organisms and their biological activities (Shehata *et al.*, 1996 a & b; Khalaf-Allah, 2009). Results revealed that, the maximum average value of surface water temperature in the fish farm was recorded during summer (28.14 ± 1.45 °C) and the minimum value (16.43 ± 1.12 °C) during winter. The present findings were in agreement with that, observed by many authors including Noor El Deen & Zaki (2010) & Mdegela *et al.* (2011). Concerning hydrogen ion concentration (pH), it was, ranged between 7.23 ± 0.30 during spring and 8.77 ± 0.47 during winter. In general, overall mean pH values were significantly higher in pond waters; this may be due to the increase of photosynthetic rate and the depletion of carbon dioxide. Water turbidity which is usually caused by suspended soil particles and/or plankton correlation to plankton abundance (Boyd, 1990). The present results showed lower visibility the ponds, this may be due to the higher accumulation of phytoplankton in the ponds. This agrees with the finding of Osman *et al.* (2010); Soltan *et al.* (2006). The highest value of total alkalinity (carbonate and bicarbonate) was observed during summer (242.67 ± 33.55 mg/l) and the lowest (176.00 ± 20.30 mg/L) during spring. This may be due to organic fertilization and the bacteria generated CO₂ from feed metabolism and manure decomposition, dissolved calcium and magnesium carbonate present in the pond sediment (Boyd, 1990). The results were nearly accordance with the study of Ezzat *et al.* (2012) whom reported that the range of total alkalinity was 282.5 to 426.5 mg / l in drain waters. In the present study, total un-ionized ammonia concentrations ranged between 0.74 ± 0.07 during spring and 1.27 ± 0.42 mg / l during summer. This may be attributed to high stock of fish, excretion of fish and decomposition of excess un-consumed feed represented another ammonia sources in ponds. On other hand, higher ammonia concentration may be due to decomposition of organic matter in water ponds. Ammonia formation depends mainly on water pH and temperature, where at high pH and water temperature, free toxic ammonia is released to critical levels. Similar observations were detected by many authors including Boyd (1990) & Konsowa (2007).

The highest infestation rate (78.82%) in *C. gariepinus* could be attributed to its feeding behavior as a carnivorous fish (bottom feeder) that assists in the transmission of more enteric parasites through its feeding on aquatic animals that harbor the infective stages of these parasites or even young infested fish. In addition, such fish are scale less; this may permit the penetration of the infective stages into the external body surface. Omeji *et al.* (2013) mentioned that, the catfish, *C. gariepinus* is mainly omnivorous, that feed on both aquatic plants and animals including copepods. The latter's act as first intermediate host of most internal parasites that infest freshwater fish (Mdegela *et al.*, 2011 & Abdel-Gaber *et al.*, 2015).

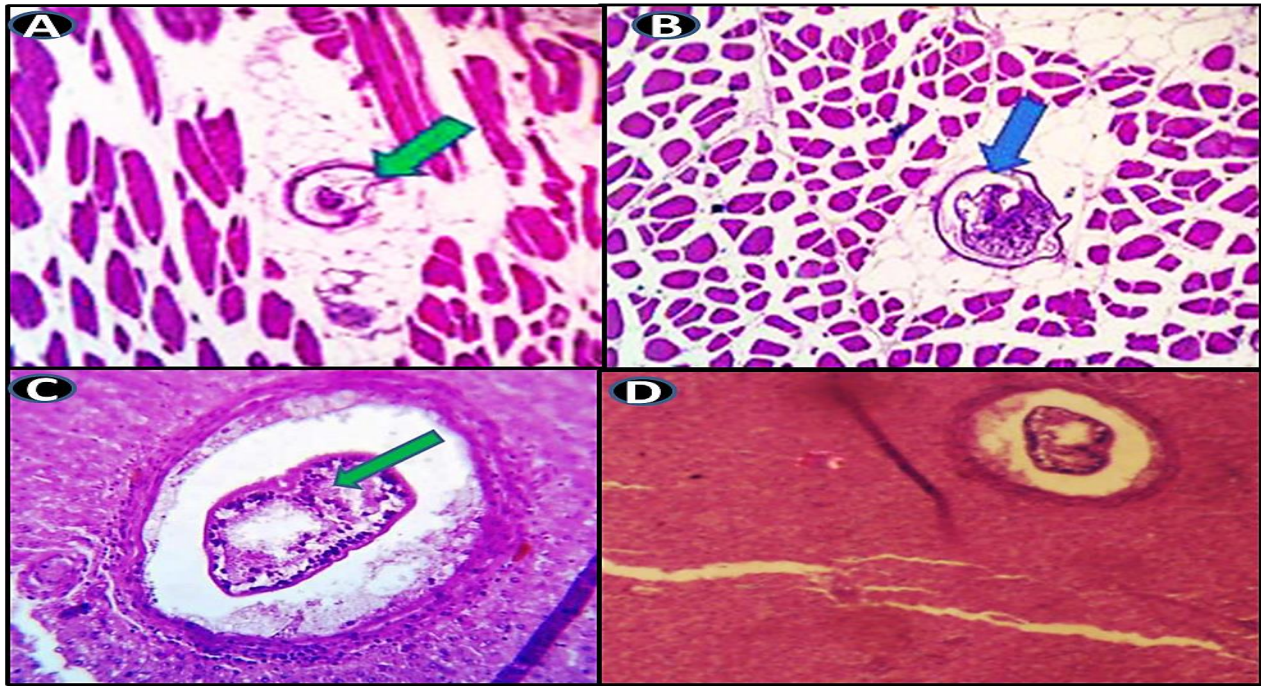


Figure 4. Photomicrograph of muscles of infected *C. gariepinus* showing (A) multiple parasitic cysts of different types (green arrow), (B) viable cyst (blue arrow) formed from scolex, (C&D) liver of the infected *C. gariepinus*, showing parasitic cyst (green arrow) (H. and E. stain- X 40, 100).

Results revealed that, the highest rate of total seasonal prevalence of parasitic infestation in *C. gariepinus*, was recorded during summer. It was followed by spring through autumn and the lowest infestation rate was recorded during winter. This may be attributed to the availability of intermediate hosts of these parasites during these seasons and increase the feeding activity in warm temperature. Similar observations were detected by many authors notably Negm El- Din *et al.* (1988) and Shager (1999). The present study exhibited that, the highest rate of parasitic infestation in different fishes was recorded in small fishes. The possible reason for this relationship may be due to that the smallest fish fed less amount of foods hence gained less immunity compared with the large ones. The present study was in agreement with Akinsanya *et al.* (2008) whom reported that smaller fish were more infected compared to the larger one, probably due to their nature of acquired immunity with age. Regarding the prevalence of parasitic infestation in relation to sex of *C. gariepinus*, the infestation rate was higher in most females than that of males.

The reason for highest prevalence of trematode 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).

Blood is a good bio-indicator of the health of any organism; it also acts as a pathological reflector of the whole body. Hence, hematological indices are important

in diagnosing the functional status of the fish (host) infested by parasites and also to evaluate the physiological state of fish. The present study exhibited that, the reduction in RBCs count, hemoglobin (Hb) value and packed cell volume (PCV) a result of the parasitic infestation that often leads to anemia, moreover, the parasites simply act as a stressor; and during primary stages of stress the PCV changes due to the release of catecholamine, which can mobilize red blood cells from spleen or induce red blood cell swelling as a result of fluid shift into the intracellular compartment. Similar results were recorded by Martins *et al.* (2004). Sabri *et al.* (2009) & Nnabuchi *et al.* (2015). The present study revealed that, the increase in WBCs count occurred as a pathological response since WBCs play a major role during infestation by stimulating the haemopoietic tissues and immune system to produce antibodies and chemical substances which work as defense agent against infection (Khurshid & Ahmad, 2012).

Enzymes are components of liver function test, which are dependable indicators of liver metabolism and wellness of the organism under test (Uboh *et al.* 2011). The present study showed that, the activities of ASAT and ALAT were significantly higher in the infected fishes when compared to the uninfected ones. Similar finding was observed by Nnabuchi *et al.* (2015) whom reported that AST, ALT and Urea showed a significant increase in clariid fishes. However, serum urea, creatinine and uric acid levels were observed to be elevated in the present study, due to parasitic infestation of fishes. Similar finding was reported by Kabir & Ovie (2011) whom mentioned that, the

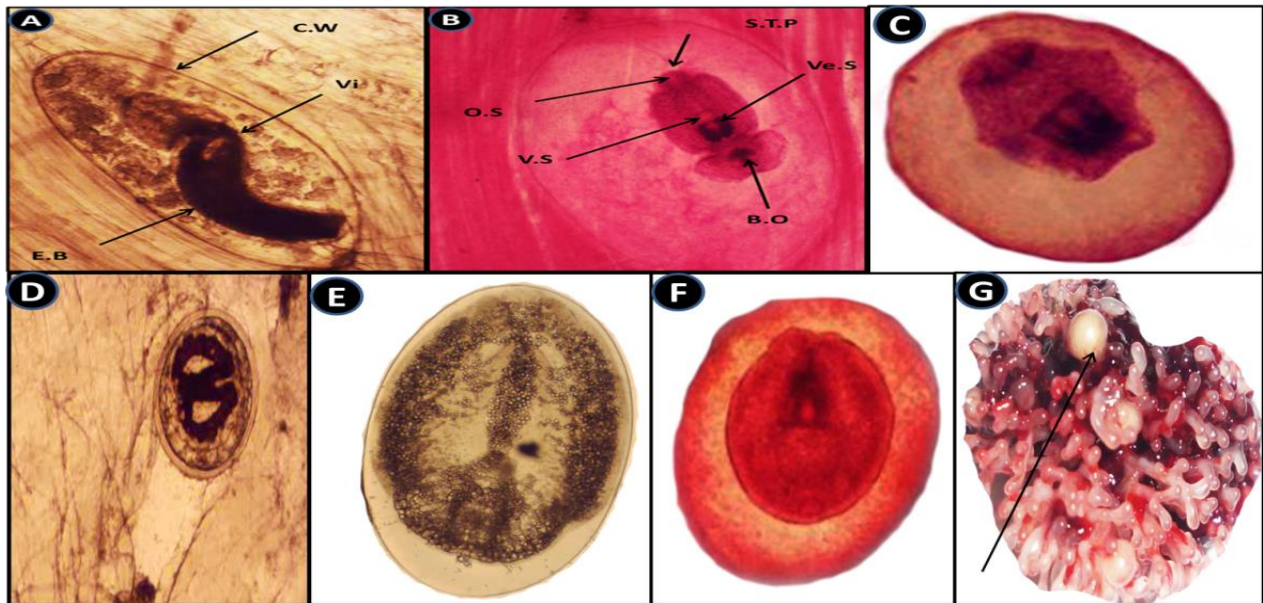


Figure 5. Photomicrographs of fresh *Diplostomum* sp isolated from the infected *C. gariepinus* (A), stained with acetic acid alum carmine (B). (C) *Pygidiopsis genata* stained with acetic acid alum carmine (D) *Prohemistomatidae*, (E) *Cyanodiplostomum azimi*, *Heterophyes* sp. stained with acetic acid alum carmine [X40] and Henneguyosis nodules in the suprabranchial organ of *C. gariepinus* (F). Showing Brandis organ (B.O), cyst wall (C.W), excretory bladder (E.B), oral sucker (O.S), stalk like process (S.T.P), vitellaria (Vi), ventral sucker (V.S) and ventrogenital sac (Ve.S) (Scale bar=50 µm).

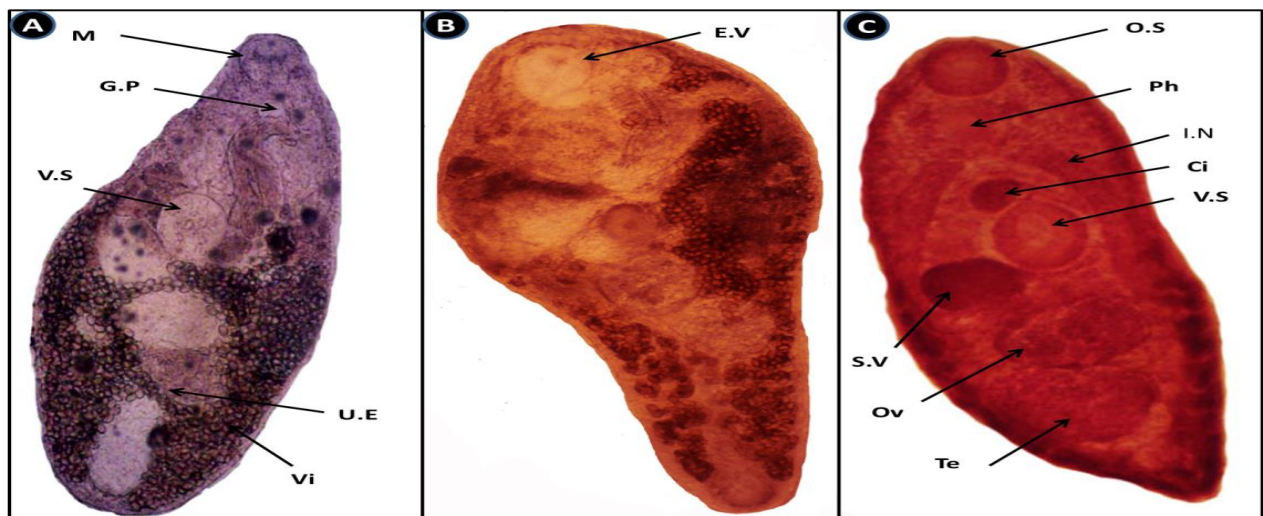


Figure 6. Photomicrographs of fresh *Orientocreadium batrachoides* isolated from the midgut of infected *C. gariepinus* (A and B) and stained with acetic acid alum (C) [X40]. Showing cirrus (Ci), excretory vesicle (E.V), genital pore (G.P), mouth (M), oral sucker (O.S), ovary (Ov), pharynx (Ph), uterus filled with eggs (U.E), seminal vesicle (S.V), testis (Te), vitellaria (Vi) and ventral sucker (V.S) (Scale bar=50 µm).

creatinine leaves the muscles and enter the blood where it is a waste product largely from the muscle breakdown. It is removed by filtration through the glomeruli of the kidney and excreted as urine. The increase in the level of creatinine in the infected fishes may be as a result of the alteration of the muscles structure of the infected fishes. The present study revealed that, the increases in blood sugar level in the infected fishes may be due to increase in the breakdown of liver glycogen or due to decreased synthesis of glycogen from glucose. Hyperglycemic condition in naturally as well as

experimentally stressed fishes may be due to impairment in the hormone level in blood involved in the carbohydrate metabolism. These findings were in agreement with Ali & Ansari (2012).

The present study revealed a decrease in the total protein and globulin values of the parasitized fish. These findings agree with McDonald & Millican (1992); Çelik & Aydın (2006) whom reported a decrease in total protein value as a result of long fasting and various distress factors. Additionally, the decrease in total protein can be attributed to the consumption of the nutrient

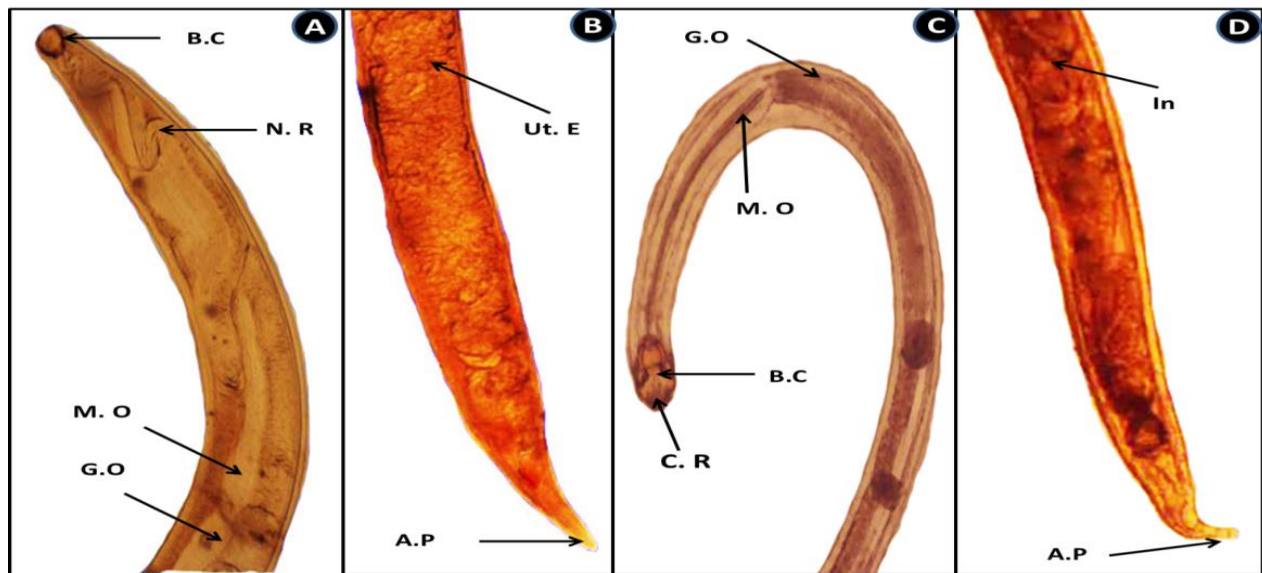


Figure 7. Photomicrographs of the anterior (A) and posterior (B) portions of *Procammallanus laevionchus* and anterior (C) and posterior (D) portion of *Paracamallanus cyathopharynx* isolated from the alimentary canal of infected *C. gariepinus*[X40]. Showing anal processes (A.P), buccal capsule (B.C), cuticular ribs (C.R), glandular oesophagus (G.O), intestine (In), muscular oesophagus (M.O), nerve ring (N.R) and uterus with eggs (Ut.E).

materials by parasites and inhibition of protein and nutrient absorption in the nutrient materials (Eissa *et al.*, 2010). Also Protein level loss arising out of cell destruction, malabsorption and fasting might be reflecting the common impact of the decreased total protein. However, Almeida *et al.* (2011) indicated that, the fish are responding to the parasite infestation by producing antibodies. Results showed that, the lowest level of A/G ratio in many fishes indicated an inflammation and liver disease. This agree with Osmani *et al.* (2009) or a shift from albumin production to globulin in response to infection as reported by Foott *et al.* (1996).

Parasites may cause irritation, injury or atrophy of tissues and occlusions of alimentary canal, blood vessels or other ducts. Their presence may lead to certain changes in the activity of enzymes, vitamins or hormones of their hosts. Also, they may introduce toxic metabolic byproducts that may lead to deprive fish from normal feeding (El-Mansy *et al.*, 2011). The histopathological examination of the liver and muscles of the infected fish indicated that, the organs most affected by infection of parasites. This is similar to the observation of Aguigwo (2002) and Omitoyin *et al.* (2006). Moreover, the cysts were found in the dermis and between muscle bundles and that in consonance with those reported by Mahdy & Shaheed (2001). The present study revealed that there was damage in liver of infected fishes. Aggregation of lymphocyte, vacuolization and dilatation of most hepatocyte, edema, disappearance of pancreatic acini and focally ruptured hepatocytes, these finding were in agreement with those obtained by Liebel *et al.* (2013) and Ali *et al.* Fish heavily infected with these parasites may experience loss of vision, reduced growth and emaciation. The

length-weight relationship (LWR) of the sampled fish indicated that the weight of the fish increased logarithmically with an increase in length, with the 'b' values lying between 2.5 and 3.5 (Seppala *et al.*, 2005).

The present study showed that, the values of 'b' of the parasitized fishes were significantly lower than 3 indicated a negative allometric growth i.e. the fish grows but is slender. Negative allometric growth is an indication of slow growth which might be due to non-availability of food as suggested by Veeramani *et al.* (2010). Similarly, the Kn values of these fishes were lower (0.94 and 1.16, respectively) than the values reported by Ndeda *et al.* (2013), which ranged between 0.98 and 3.41. Non-parasitized fish, however, showed 'b' values nearest 3 implying a slightly negative allometric growth. The rapid growth might be an indication of abundant food supply and other favorable conditions as suggested by Froese (2006).

In conclusion, the presence of parasitic infection in this study is enough to cause hematological, biochemical indices and pathological effect in fishes by reducing their growth, development and even the market. This requested for levitation awareness in fish health management and application of suitable control measures.

Ethical Statement

The conducted experiment was held according to the European Union Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. All procedures were monitored and approved by the Bioethical Committee of Al-Azhar University.

Funding Information

Not applicable / No funding was received.

Author Contribution

Mahmoud Radwan: Conceptualization, formal analysis, investigation, methodology, validation, writing - original draft writing - review & editing.

Sabry Shehata: Investigation, methodology, validation, writing - original draft. conceptualization, formal analysis, resources, writing - original draft.

Yasser Abdelhad: writing - original draft.

Ramadan Mohammed: Conceptualization, investigation, methodology, writing, writing - review & editing.

Mohamed Hamed: writing - original draft.

Mohammad Magdy: Writing - original draft, writing - review & editing.

Conflict of Interest

The authors declare no conflicts of interest.

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