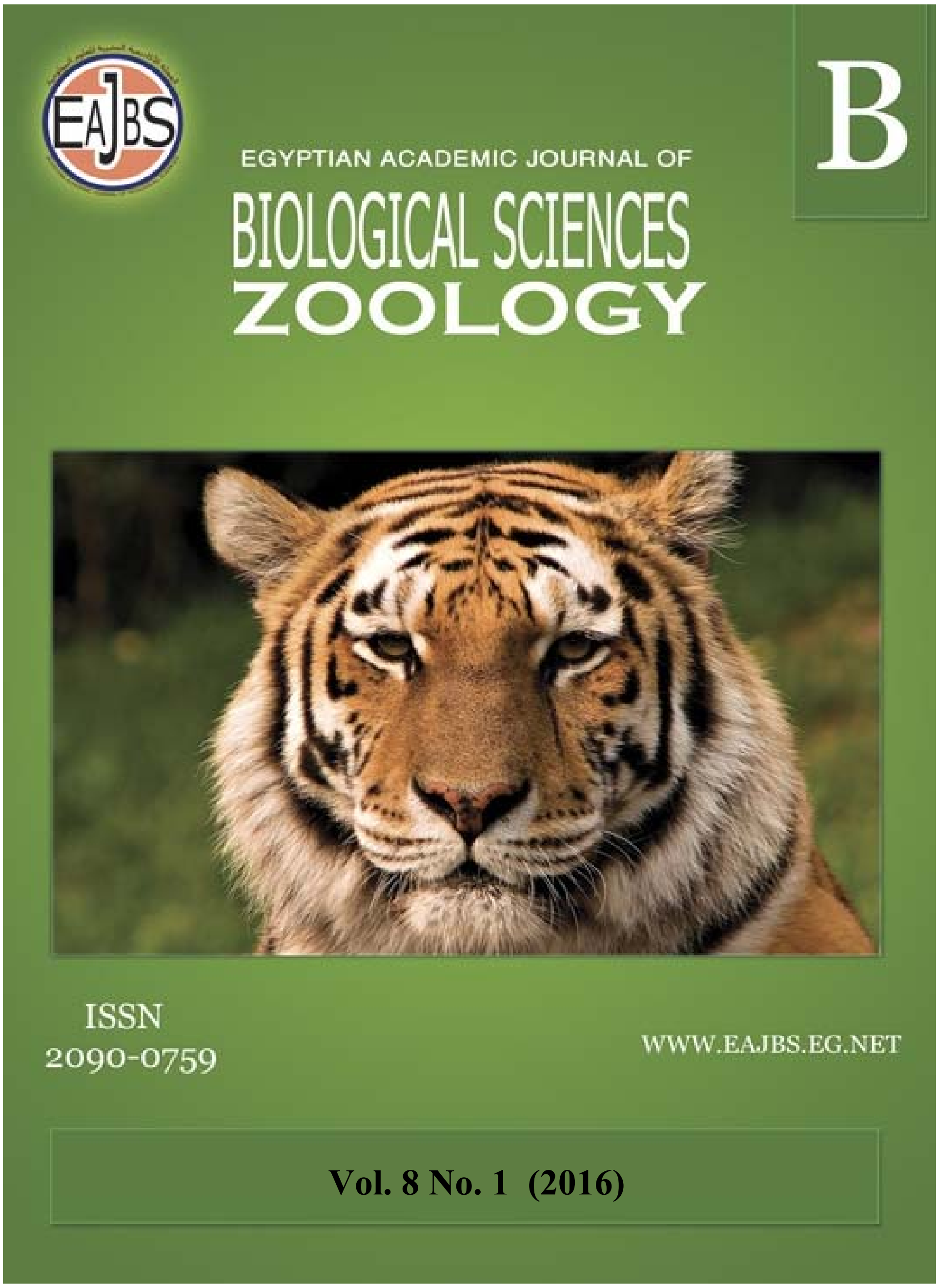

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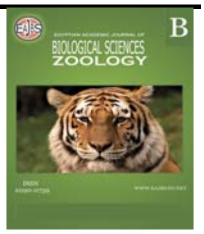

Comparative Study of the Effect of Biological Factors on Helminthes Occurrence in *Oreochromis niloticus* and *Tilapia zilli* from Lake Manzala, Port Said, Egypt.

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ABSTRACT

The aim of this work is to study the effect of host biological factors on the different parasitic helminthes in *Oreochromis niloticus* and *Tilapia zilli* from Lake Manzala. The sex, weight and length of fish were determined. The examination of fish revealed the presence of seven distinct defined metacercariae in addition to undifferentiated metacercariae and nematode larvae. The recovered metacercariae were *Centrocestus sp., Haplorchis sp., heterophyes sp., Stictodora sp., Pygidiopsis sp., Echinostoma sp.* and *Phagicola sp.* Results showed that *O. niloticus* was more susceptible to parasitic infection than *T. zilli*. The high est prevalence of infection was found in larger fish (weight and length) and in males (Sex) in the case of *O. niloticus*, while the highest prevalence of infection was found in smaller fish (weight and length) and in females (sex) in *T. zilli*. The occurrence of each of the helminthes gives a different response to these factors as discussed in this work.

INTRODUCTION

Oreochromis niloticus and *Tilapia zilli* are important sources of protein food for the Egyptians. It provides about 70% of the total fish production in Egypt (EI-Zarka, 1961). Digenetic trematodes and their metacercariae take a great interest as they were one of the most widely distributed parasites infecting fish causing low weight gain, high mortality and some of them may have zoonotic importance (Hernandez *et al*., 1998).

Biotic factors as parasite life cycles and fish age/size and abiotic factors such as water temperature, current and/or depth also affect the distribution of parasites which may be aggregated or over dispersed in the host (Iyaji and Amana, 2015).

Ramadan (1991) reported that the host species affect the rate of infection. He recorded the rate of infection in *T. nilotca, T .zilli* and *T. galilaea* fish in Lake Manzala. The highest rate of infection was found in *T. zilli* while, the lowest rate of infection was found in *T. nilotica*.

Reimchen and Nosil (2001) reported that fish males and females can differ in the levels of the infection and such differences may be mediated by the costs of sexual selection or by ecological differences between the different genders. Overall parasite prevalence was greater in males than in females. However, this excess did not occur for each species of the parasite.

Hassan *et al*. (2012) reported that the prevalence, intensity and abundance of each metacercaria in *T. zilli* species showed different responses to the different factors including sex, weight and length of the host.

The aim of this work is to study the effect of host biological factors on the helminthes infecting *O. niloticus* and *T. zilli* from Lake Manzala “Port said market”.

MATERIALS AND METHODS

30 specimens of *O. niloticus* and 30 specimens of *T. zilli* were collected from Lake Manzala in Port Said Governorate in ice boxes and transported to the laboratory for parasitological examination. The total length of fish were determined by measuring the distance from the tip of the longest jaw or the end of stout to the longest caudal lobe or end pushed together(Miller and Lea, 1982).*O. niloticus* species were divided into two length classes; as follows: Class 1 (<16cm) and Class 2 (>16cm). *T. zilli* species were divided into two length classes; as follows: Class 1 (<10cm) and Class 2 (>10cm).

The weights of the collected fish species were determined using (CY) balance. *O. niloticus* species were divided into two weight classes; as follows: Class 1 (<32g) and Class 2 (>32g). *T. zilli* species were divided into two weight classes; as follows: Class 1 (<16g) and Class 2 (>16g).

Fish sex was determined according to Guerrero and Shelton (1974). Body cavity was opened to determine the sex, and the gonads were isolated. Small pieces of the gonads were placed on a glass slide, then pressed (squashed) with a glass cover slip and observed under dissecting microscope.

The muscles and the internal viscera including liver, kidney, gonads and digestive tract t were examined for the possible presence of trematode metacercariae (MC). These organs were kept in saline solution for few minutes for possible recover of any parasites. Tissues were screened for the presence of MC by compression method in which 0.1 gm were taken from muscles and visceral organs. Each piece was compressed between two microscopic glass slides and examined for the presence of MC (Sayasone *et al*., 2007; and Elsheikha and Elshazly, 2008).

Metacercariae were separately sorted out according the general features and were tentatively identified to genus level based on different morphological details; the shape of the cysts, site of infection and shape, position and contents of the execratory bladder under a light microscope (Amer, 1996; Saad, 2007; Elsheikha and El-shazly, 2008; Sohan *et al*., 2009; Taher, 2009; Pinto and De Melo, 2012 and Motarjemi *et al*., 2013).

The prevalence, mean intensity and abundance of recovered helminthes according to each factor considered were calculated according Margolis *et al*. (1982). To satisfy the assumption of the statistical analysis used, all the data were normalized by log (x+1) transformation to achieve linearity. For studying the significant differences between groups, analysis of variance was used. All data were analyzed with the software packages Microsoft SPSS version 15.0, for statistical evaluation. Values of $P < 0.05$, $P < 0.01$and 0.001 reflected levels of significance.

RESULTS AND DISCUSSION

The examination of fish revealed the presence of seven distinct defined metacercariae in addition to undifferentiated metacercariae and nematode larvae. The recovered metacercariae were *Centrocestus sp., Haplorchis sp., heterophyes sp., Stictodora sp., Pygidiopsis sp*., *Echinostoma sp.* and *Phagicola sp.* Nematode larvae and *Phagicola sp.* were specific for *T. zilli* while, *heterophyes sp.* was specific for *O. niloticus*.

The highest total prevalence was recorded in *O. niloticus* 86.7% higher than in *T. zilli* 83.7%. Regarding prevalence of different species of helminthes, the highest prevalence was recorded in both species for undifferentiated metacercariae followed by *Centrocestus sp*. The total abundance was higher in *O. niloticus*1527. 3±461.1 than in *T. zilli* 674.8±214.7, the highest abundance was recorded in both species for undifferentiated metacercariae followed by *Centrocestus sp*. in *O. niloticus* and *Echinostoma sp.* in *T. zilli*. The total intensity was higher in *O. niloticus*1762.3±517.6 than in *T. zilli* 788.8±241.9, the highest intensity was recorded in both species for undifferentiated metacercariae followed by *Centrocestus sp*. in *O. niloticus* and *Haplorchis sp.* in *T. zilli*. Regarding prevalence, abundance and intensity with different species of helminthes are shown in Table (1).

Table 1: Prevalence, mean abundance (±SE) and mean intensity (±SE) of helminthes infecting *O. niloticus* and *T. zilli* of Lake Manzala according to host species.

Helminthes	**Prevalence**		**Mean abundance**		**Mean intensity**	
	O. niloticus	***T. zilli***	***O. Niloticus***	***T. Zilli***	***O. niloticus***	***T. Zilli***
Centrocestus sp.	60%	56.3%	307. 3±115.4	75.2±24.7	512.2±177.97	395±135
Haplorchis sp.	40%	50%	88±33.6	80±26.3	395±134.9	450±190
Heterophyes sp.	33.3%	0	60±24.01	0	202±74.6	0
Stictodora sp.	50%	33.3%	32.7±9.5	51.4±20.4	124±23.6	149±46.3
Echinostoma sp.	20%	36.7%	7.7±5.4	84.1±40.8	38.3±24.4	221.8±96.1
Pygidiopsis sp.	6.7%	30%	11.3±9.2	24.5±11.3	170±100	78.9±29.9
Phagicola sp.	0	26.7%	0	22.4±12.8	0	81.3±40.49
Undifferentiated metacercariae	86.7%	83.7%	1020.3±341.8	336.6±129.6	3437.1±925.4	2013.3±686.1
Nematode larvae	0	6.7%	0	10 n=2	0	10 n=2
Total	86.7%	83.7%	1527. 3±461.1	674.8±214.7	1762.3±517.6	788.8±241.9

n = number of infected fish.

In our study *O. niloticus* was more susceptible to parasitic infection than *T. zilli* and this agrees with (Taher, 2009) who recorded that the rate of prevalence in *O. niloticus* and *T. zilli* collected from Assiut Governorate was 84.75%, and 78.25%, respectively. Ramadan, 1991 recorded that the highest percentage of infection was found in *T. zilli* 67.6% and the lowest in *O. niloticus* 47.4% this variation may be due to a multiple reasons such as host species, different habits, food type, the presence and absence of snails and birds which plays an important role in the life cycle of many helminths and also the differences in the seasons in which the samples were collected.

The effect of fish length on the prevalence, abundance and intensity of different helminthes infecting *O. niloticus* and of *T. zilli* is shown in Table (2).

In *O. niloticus*, the results showed that prevalence was the highest (84.2%) in fish within length class 2 (>16) and lowest (81.8%) in fish within length class 1 (<16), while, in the case of *T. zilli* the prevalence was the highest (90%) in fish within length class 1 (<10) and the lowest (70%) in fish within length class 2 (>10). Malek and

Mobedi (2001) stated that the decrease in the prevalence of infection in the larger fish could be a result of the increase in resistance to diseases of the host with the increase in length and differ of feeding habit of the host. Similar view is confirmed by Grutter *et al.* (2002).

Table 2: Prevalence, mean abundance (±SE) and mean intensity (±SE) of helminthes infecting *O. niloticus* and *T. zilli* of Lake Manzala according to host length.

Helminthes	prevalence				Mean abundance				Mean intensity
	O. niloticus		*T. zilli*		*O. niloticus*		*T. zilli*		*O. niloticus*
	Class 1 (N=11)	Class2 (N=19)	Class 1 (N=20)	Class2 (N=10)	Class 1 (N=11)	Class2 (N=19)	Class 1 (N=20)	Class 2 (N=10)	Class 1 (N=11)
Centrocestus sp.	45%	68.4%	50%	60%	243.6±204.2	344.2±142.3	56.5±23.4	105±55.5	243.6±204.2
Haplorchis sp.	18.2%	52.6%	50%	50%	14.6±12.7	130.5±50.4	85.5±35.6	61±30.2	14.6±12.7
Heterophyes sp.	9.09%	47.4%	0	0	90 n=1	90±36.2	0	0	90 n=1
Stictodora sp.	36.4%	58.89%	35%	30%	7.3±3.6	47.4±13.9	40±19.9	69±45.4	7.3±3.6
Echinostoma sp.	0	31.6%	30%	50%	0	12.1±8.4	39±16	166±113.3	0
Pygidiopsis sp.	0	10.5%	45%	0	0	17.9±14.5	35.5±15.8	0	0
Phagicola sp.	0	0	30%	20%	0	0	19.5±14.98	26±22.9	0
Undifferentiated Metacercariae	81.8%	78.95%	20%	70%	85.5±30.1	1561.6±502.5	269±108.3	453±316.1	85.5±30.1
Nematode larvae	0	0	0	20%	0	0	0	20 (n=2)	0
Total	81.8%	84.2%	90%	70%	359.1±215.3	2203.7±675.7	545±177	882±527.3	359.1±215.3

N = number of examined fish, n = number of infected fish.

In *O. niloticus*, the highest total mean abundance of infection (2203.7±675.7) was recorded in fish within length class 2 (>16) and the lowest (359.1±215.3) in fish within length class 1 (<16) (P<0.001) and in *T. zilli* the highest total mean abundance of infection (882±527.3) was recorded in fish within length class 2 (>10) and the lowest (545±177) in fish within length class 1 (<10). In this work there was a direct relationship between abundance and the host length and this agrees with Blackburn and Gatson (1997) who recorded relationship between parasite abundance and body total size, this in turn may be attributed to the more specialized immunity of these fish with size and age.

In *O. niloticus,* the highest total mean intensity (2616±760.6) was recorded in fish within length class 2 (>16) and the lowest (395±234.7) in fish within length class1 (<16) (P<0.001) and the same in *T. zilli* as the highest total mean intensity of infection (1461.7±816.2) was recorded in fish within length class 2 (>10) and the lowest (794±307.7) in fish within length class1 (<10) (P<0.05). This disagrees with Hassan (2012) who found that the total mean intensity of infection is inversely proportional to *T. zilli* and *O. niloticus* length.

The effect of fish weight on the prevalence, abundance and intensity of different helminthes infecting *O. niloticus* and of *T. zilli* is shown in Table (3).

In *O. niloticus*, the results showed that prevalence was the highest (100%) in fish within weight class 2 (>32) and the lowest (83.3%) in fish within weight class 1 (<32) and reversibly in *T. zilli* as the prevalence was the highest (95.8%) in fish within weight class 1 (<16) and lowest (55.6%) in fish within weight class 2 (>16) and this may be due to the immune response as discussed previously.

In *O. niloticus*, the highest total mean abundance of infection (5241.7±1285.83) was recorded in fish within weight class 2 (>32) and the lowest (598.8±240.2) in fish within weight class 1 (<32) (P<0.001) and this also repeated in *T. zilli* as the highest total mean abundance of infection (688.9±585.2) was recorded in fish within weight class 2 (>16) and the lowest (643.8±176.03) in fish within weight class 1 (<16).

Table 3: Prevalence, mean abundance (±SE) and mean intensity (±SE) of helminthes infecting *O. niloticus* and *T. zilli* of Lake Manzala according to host weight.

Helminthes	prevalence				Mean abundance				Mean intensity			
	O. niloticus		*T. zilli*		*O. niloticus*		*T. zilli*		*O. niloticus*		*T. zilli*	
	Class 1 (N=24)	Class2 (N=6)	Class 1 (N=21)	Class2 (N=9)	Class 1 (N=24)	Class2 (N=6)	Class 1 (N=21)	Class 2 (N=9)	Class 1 (N=24)	Class2 (N=6)	Class 1 (N=21)	Class 2 (N=9)
Centrocestus sp.	50%	100%	57.1%	44%	161.7 ±95.8	890 ±363.97	68.6 ±24.06	82. 2±59.7	323.3 ±183.3	890 ±363.97	120 ±35.8	185 ±122.6
Haplorchis sp.	25%	100%	57.1%	33.3%	20±11	360 ±109.3	96.7 ±33.9	32.2 ±28.6	80 ±35.6	360 ±109.3	238.6 ±74.4	135 ±125
Heterophyes sp.	16.7%	83.3%	0	0	13.8 ±7.6	245 ±84.2	0	0	66 ±27.3	294 ±83.9	0	0
Stictodora sp.	37.5%	100%	38.1%	22.2%	13.8 ±6.5	108.3 ±20.2	57.1 ±25.5	32.2 ±29.8	36.7 ±14.9	108.3 ±20.2	165.7 ±59.4	145 ±125
Echinostoma sp.	16.7%	33.3%	38.1%	33.3%	8.3±6.7	5±3.4	65.7 ±30.3	117.8 ±115.3	50 ±36.7	15±5	140 ±34.2	353.3 ±404.1
Pygidiopsis sp.	0	33.3%	42.9%	0	0	56.7 ±44.2	33.8 ±15.1	0	0	170 ±100	132 ±40.3	0
Phagicola sp.	0	0	33.3%	11.1%	0	0	29.5 ±17.4	30 (n=1)	0	0	88.6 ±46.5	30 (n=1)
Undifferentiated Metacercariae	83.3%	100%	95.8%	55.6%	381.3 ±181.4	3576.7 ±1071.98	291.9 ±103.4	420 ±355.2	352.5 ±172.4	3365 ± 1068.9	304 ±107.8	756 ±625.04
Nematode larvae	0	0	4.8%	11.1%	0	0	10 n=1	10 n=1	0	0	10 (n=1)	10 (n=1)
Total	83.3%	100%	95.8%	55.6%	598.8 ±240.2	5241.7 ±1285.83	643.8 ±176.03	688.9 ±585.2	718.5 ±281.6	2616.9 ±760.6	880 ±266.95	1537.5 ±1273.9

N = number of examined fish, n = number of infected fish.

In *O. niloticus*, the highest total mean intensity of infection (2616.9±760.6) was recorded in fish within weight class 2 (>32) and the lowest (718.5±281.6) in fish within length class1 (<32) (P< 0.001) this agrees with Bell and Burt (1991), Machado *et al*. (1994), Takemoto and Pavanelli (1994), Poulin (1995), Fiorillo and Font (1996) and luque *et al.* (1996 a&b) who found that increased size and age of fish result in significant increase in the levels of parasitism. This also occurred in *T. zilli*as the highest total mean intensity of infection (1537.5±1273.9) was recorded in fish within weight class 2 (>16) and the lowest (880±266.95) in fish within length class1 (<16).

The effect of fish sex on the prevalence, abundance and intensity of each of the different helminthes infecting *O. niloticus* and of *T. zilli* is shown in Table (4).

Table 4: Prevalence, mean abundance (±SE) and mean intensity (±SE) of helminthes infecting *O. niloticus* and *T. zilli* of Lake Manzala according to host sex.

Helminthes	prevalence				Mean abundance				Mean intensity			
	O. niloticus		*T. zilli*		*O. niloticus*		*T. zilli*		*O. niloticus*		*T. zilli*	
	Male (N=12)	Female (N=18)	Male (N=14)	Female (N=16)	Male (N=12)	Female (N=18)	Male (N=14)	Female (N=16)	Male (N=12)	Female (N=18)	Male (N=14)	Female (N=16)
Centrocestus sp.	**75%**	**50%**	**35.7%**	**68.8%**	**520.8 ±237.8**	**165 ±102.4**	**40±24.2**	**101.3 ±39.1**	**694.4 ±297.6**	**330 ±194.3**	**112 ±57.5**	**147.3 ±51.5**
Haplorchis sp.	**41.7%**	**38.8%**	**42.9%**	**56.3%**	**159.2 ±72.6**	**40.6 ±24.5**	**48.6 ±20.8**	**102.5 ±44.1**	**382 ±116.1**	**104.3 ±57.1**	**170 ±35.1**	**238.3 ±93.8**
Heterophyes sp.	**41.7%**	**27.8%**	**0**	**0**	**88.3±44.5**	**41.1 ±27.1**	**0**	**0**	**212 ±80.7**	**148 ±85.2**	**0**	**0**
Stictodora sp.	**50%**	**50%**	**21.4%**	**43.8%**	**29.2±13.5**	**35±13.4**	**17.9 ±14.3**	**77.5 ±33.99**	**58.3 ±21.4**	**70 ±21.3**	**83.3 ±58.97**	**200 ±66.3**
Echinostoma sp.	**8.3%**	**27.8%**	**28.6%**	**43.8%**	**10 (n=1)**	**12.2±8.8**	**27.1 ±17.01**	**128.8 ±71.6**	**10 (n=1)**	**44 ±29.1**	**145±85**	**238.3 ±93.8**
Pygidiopsis sp.	**8.3%**	**5.6%**	**21.4%**	**31.3%**	**270 (n=1)**	**70 (n=1)**	**29.3 ±19.4**	**18.8 ±11.9**	**270 (n=1)**	**70 (n=1)**	**195 ±45**	**90 ±50.3**
Phagicola sp.	**0**	**0**	**35.7%**	**18.8%**	**0**	**0**	**25.7±21.2**	**18.1 ±14.5**	**0**	**0**	**72 ±57.1**	**96.7 ±67.7**
Undifferentiated Metacercariae	**91.7%**	**83.3%**	**64.3%**	**100%**	**1025 ±468.1**	**1017.2 ±487.5**	**16±1.1**	**508.1 ±218.9**	**1118.2 ±502.6**	**1220.7 ±573.2**	**197.8 ±115.8**	**7.7±1.4**
Nematode larvae	**0**	**0**	**7.1%**	**6.3%**	**0**	**0**	**10 n=1**	**10 n=1**	**0**	**0**	**10 (n=1)**	**10 (n=1)**
Total	**91.7%**	**83.3%**	**64.3%**	**100%**	**1845.8 ±708.1**	**1315 ±617.6**	**316.4 ±141.4**	**955.6 ±359.4**	**2013.6 ±753.5**	**1578 ±725.4**	**448 ±248.5**	**1315.5 ±489.5**

N = number of examined fish, n = number of infected fish.

In *O. niloticus*, Results showed that the prevalence with metacercariae was higher in male specimens (91.7%) than in female ones (83.3%) and reversibly in *T. zilli* as the prevalence was the highest in female specimens (100%) than in male ones (64.3%). Comparing abundance values showed that the total mean abundance of infection was higher in males (1845.8±708.1) than females (1315±617.6) in *O. niloticus* while, infection was higher in females (955.6±359.4) than males (316.4±141.4) in *T. zilli*. Finally the intensity values showed that the total mean intensity of infection was higher in males (2013.6±753.5) than females (1578±725.4) (P<0.001) in *O. niloticus* and reversibly in *T. zilli* as the intensity was the highest in female specimens (1315.5±489.5) than in male ones (448±248.5).

Emere (2000) revealed differences in infestation between males and females to differences in feeding which could be in terms of quality and quantity. It could also be attributed to differences in the degree of resistance to infestation. Males are usually more sensitive to the parasites than females partially due to testosterone synthesis, which might cause decreasing immune competency (Gbankoto *et al.*, 2001).

In conclusion, *O. niloticus* was more susceptible to parasitic infection than *T. zilli* and the incidence of parasitic infections recorded in the present study was markedly high and showed different responses to the biological factors that varied according to parasite species and fish host.

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