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Some factors affecting metacercarial infections in *Tilapia zilli* from Lake Timsah, Ismailia, Egypt

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ABSTRACT

The objective of this work is to study some environmental and biological factors affecting metacercarial infections in *Tilapia zilli* from two sites at Lake Timsah, Ismailia, Egypt. Fish were collected monthly from two sites during a period from March 2009 to February 2010. Fish length and sex were determined. Five different trematode metacercariae were recovered from *T. zilli* fish, namely, *Euclinostomum* sp., *Pygidiopsis* sp, *Phagicola sp.*, and *Stictodora* sp. and some were undifferentiated. Infection was higher in site 2 (42.4%) than site 1 (32.59%). The highest infection was recorded in winter (79.76%), and the lowest in spring (9.52%). It was the highest (40.51%) in medium-sized fish. Males were more infected (38.93%) as compared to females (30.04%). Prevalence, intensity and abundance of each metacercaria species showed different responses to the considered factors as discussed in this work.

Keywords: *metacercariae, Tilapia zilli, sites, seasonal patterns, host size and sex.*

INTRODUCTION

Parasites form a vital part of biotic communities within aquatic ecosystems (Mouritsen and Poulin, 2002) and may account for a substantial proportion of the overall biomass (Kuris *et al.*, 2008). They have to be considered as an important determinant in ecosystem functioning by directly affecting specific physiological host functions (Gorbushin, 1997), thus altering host population dynamics and influencing community structure (Mouritsen and Poulin, 2005).

Digenean trematodes are the dominant metazoan parasites of macroinvertebrates inhabiting the areas. The life cycles of these endoparasites generally include the exploitation of different host organisms' zone (Mouritsen and Poulin, 2002). Digenetic trematodes and their metacercariae (MC) have taken a great interest in most countries especially for the human care against the transmissible diseases (Taher, 2009). Metacercariae infections cause low weight gain, high mortality, immarketability of the infected fish and some of these parasites may have zoonotic importance (Hernandez *et al*., 1998).

The influence of biotic and abiotic factors such as host condition and seasonal variation on transmission of fish parasites in terms of their prevalence and intensity is well-documented, factors affecting occurrence and population dynamics of heterophyid MC in fish are far less characterized (Sithithaworn *et al.*, 1997 and Elsheikha and Elshazly, 2008), especially those factors related to co-infections. The pattern of seasonal variation and effect of biotic factors on heterophyid MC could be of considerable importance in planning for parasite control (Sithithaworn *et al.*, 1997).

Metcercarial infections are the most common parasitic infection of the fish in Egypt. Despite the importance of metacercriae in the life cycle of heterophyids, surprisingly little is known about their epidemiology and habitat in their natural hosts in Egypt. Hence, the objective of this study is to study the environmental and biological factors affecting metacercarial infections in *T. zilli* from Lake Timsah, Ismailia, Egypt.

MATERIAL AND METHODS

Fish were collected from Lake Timsah in Ismailia city during a period extended from March 2009 to February 2010. Two sites were selected in Lake Timsah for this study, site 1 (N 30° 35' 10.78" E 23° 16' 19.19") and site 2 (N 30° 34' 44.45" E 32° 17' 21.10"). Altogether, 260 specimens of the cichlid *T. zilli*, were collected from Lake Timsah. The fish were transported immediately to the laboratory to be examined. The total length of *T. zilli* was determined by measuring the distance from the tip of the longest jaw or the end of the snout to the longest caudal lobe pushed together (Miller and Lea, 1976). *T. zilli* fish were divided into three length classes; Class 1 (<11cm.), class 2 (<13 cm.) and class 3 (>13 cm.). Fish sex was determined according to Guerrero and Shelton (1974). Body cavity was opened to determine the sex, and gonads were isolated.

Body cavity was opened and the internal viscera including the muscles, liver, and kidney 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 snipes were taken from muscles and visceral organs such as liver, kidney and gonads. 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 collected by the general feature and were tentatively identified to genus level based on the morphological details, their dimensions, the shape of cysts, site of infection, presence of suckers, and shape and contents of excretory bladder under a light microscope (Amer, 1996; Elsheikha and Elshazly, 2008; and Sohn *et al.*, 2009). In addition, the characteristic occurrence of different heterophyid MC in fish either singly or in groups was considered for confirming MC identification (Elsheikha and Elshazly, 2008).

The mean prevalence, intensity and abundance of metacercarial infection in fish muscles were recorded according to Margolis *et al.* (1982). To satisfy the assumption of statistical analysis used, all the data were normalized by log (x+1) transformation to achieve homoscedasticity or linearity. For studying the 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 $P < 0.001$ reflected levels of significance.

RESULTS AND DISCUSSION

Five different trematode MC were recovered from *T. zilli* fish, and they are, *Euclinostomum* sp., *Pygidiopsis* sp, *Phagicola* sp., and *Stictodora* sp. as well as undifferentiated Heterophyid MC. The effect of site variation on the prevalence of *T. zilli* in the two different sites in Lake Timsah is shown in table (1). The highest total prevalence was recorded in site 2 (42.4%) higher than in site 1 (32.59%). Regarding prevalence of different species of MC, the highest prevalence was recorded in both sites for *Phagicola* sp. The total mean abundance of infection was higher in site 1

(34.67±2.4) than in site 2 (26.16±1.8) (P < 0.05) (Table 1). Regarding infection with different species of MC, the highest abundance of infection was recorded in site 1 by *Clinostomum* sp. (17.41±2.3) (P < 0.01). In site 2, the highest abundance of infection was also recorded for *Euclinostomum* sp. (10.64±1.9) (P < 0.001). The total mean intensity of infection was higher in site 1 (106.36±9.77) than in site 2 (61.70±6.2) (P<0.01) (Table1). Regarding infection with different species of MC, the highest intensity of infection was in average recorded in site 1 for *Euclinostomum* sp. (123.68±8.2) (P < 0.001). In site 2, the highest intensity of infection was recorded for undifferentiated heterophyid MC (90.00±7.22) (P < 0.001). The present study suggests that such variation in prevalence may be related to the difference in the habitat, food supply, abundance of both aquatic snails (the intermediate host), and the aquatic piscivorous birds, which play the main role to complete the life cycle of some digenetic trematodes (Taher, 2009).

Table1: Prevalence, mean abundance (±SE) and m intensity (±SE) of metacercariae infecting *T. zilli* from two sites of Lake Timsah.

Metacercariae species	Prevalence of metacercariae			Abundance			Intensity		
	Site 1 (N = 135)	Site 2 (N = 125)	All sites (N = 260)	Site 1	Site 2	All sites	Site 1	Site 2	All sites
Undifferentiated Heterophyid MC	2.22 % (n=3)	3.2% (n=4)	2.69% (n=7)	1.48±0.88	2.88±1.73	2.15±0.95	66.67±6..33	90.00±7.22	80.00±8.4
***Euclinostomum* sp.**	14.07% (n=19)	12% (n=15)	13.08% (n=34)	17.41±2.3	10.64±1.9	14.15±2.5	123.68±8..2	88.67±5.85	108.24±11.34
***Pygidepsis* sp.**	2.96% (n=4)	4.8% (n=6)	3.85% (n=10)	2.96±1.61	0.64±0.27	1.85±0.85	100.00±8..47	13.3±2.13	48.00±4.2
***Phagicola* sp.**	19.26% (n=26)	19.2% (n=24)	19.23% (n=50)	9.04±2.55	9.76±1.59	9.38±2.17	46.92±3.2	48.80±5.46	47.84±4.5
***Stictodora* sp.**	8.15% (n=11)	9.6% (n=12)	8.85% (=ى23)	4.07±1.74	2.16±0.73	3.15±0.97	45.83±5.49	23.33±4.32	35.65±8.5
Total prevalence	32.59% (n=44)	42.4% (n=53)	37.31 % (n=97)	34.67±2.4	26.16±1.8	30.58±1.9	106.36±9.77	61.70±6.2	81.96±8.99

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

The epidemiological picture of food borne trematodiasis has changed in recent years, in some setting, the prevalence of food borne trematode infections increases while in other it decreases significantly, which can be explained by factors such as social, economic development, urbanization, adequate food inspections, health, education campaigns, use of chemical fertilizers and water pollution (Keiser and Utzinger, 2004).

The effects of seasonal variation on the prevalence, abundance and intensity of infection in *T. zilli* are shown in table (2). The highest total prevalence was recorded in winter (79.76%), followed by summer (53.33%), autumn (25%), and spring (9.52%). In spring, the only infection was recorded for *Pygidiopsis* sp. (8.33%). In summer, the highest prevalence was recorded for *Euclinostomum* sp. (28.33%). In autumn, the highest prevalence was recorded for *Stictodora* sp. (15%), and no infection was recorded in case of *Pygidiopsis* sp. In winter, *Phagicola* sp. (62.5%) was the prevalent and the least recorded was *Euclinostomum* sp. (26.79%). undifferentiated heterophyid MC, *Pygidiopsis* sp., and *Stictodora* sp. were absent. The intensity of infection was recorded in spring for *Pygidiopsis* sp. (48.57±12.05). In summer, the highest intensity of infection was recorded for undifferentiated heterophyid MC (91.67±13.39) (P < 0.001). In autumn, the intensity of *Stictodora* sp. (23.33±3.33) was the leading (P < 0.01). In winter, it was recorded for *Euclinostomum* sp. (166.0±10.38) and *Phagicola* sp. (43.06±9.39) (P < 0.001).

Table 2: Prevalence, mean abundance (±SE) and m intensity (±SE) of metacercariae infecting *T. zilli* of Lake Timsah durin

etacercariae species	Prevalence of metacercariae				Abundance				Intensity			
	Spring (N = 84)	Summer (N = 60)	Autumn (N = 60)	Winter (N = 56)	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Undifferentiated Heterophyid MC	0%	10% (n=6)	1.67% (n=1)	0%	0	9.17±4.0	0.17 (n=1)	0	0	91.67±13.39	0	0
***Euclinostomum* sp.**	0%	28.33% (n=17)	3.33% (n=2)	26.79% (n=15)	0	19.33±2.87	0.50±0.37	44.46±5.01	0	68.24±12.35	15.0±5.0	166.0±10.38
***Pygidepsis* sp.**	8.33% (n=7)	5% (n=3)	0%	0%	4.05±2.38	2.33±1.51	0	0	48.57±12.05	46.67±6.47	0	0
***Phagicola* sp.**	0%	15% (n=9)	10% (n=6)	62.5% (n=35)	0	13.67±3.51	1.17±0.48	27.68±3.62	0	91.11±11.14	11.67±1.67	43.06±9.39
***Stictodora* sp.**	0%	23.33% (n=14)	15% (n=9)	0%	0	10.17±3.90	3.50±1.18	0	0	43.57±7.39	23.33±3.33	0
Total prevalence	8.33% (n=7)	53.33% (n=32)	25% (n=15)	79.76% (n=43)	4.05±2.38	54.67±5.89	5.33±1.45	71.61±6.83	48.57±12.05	102.50±12.3	21.33±3.36	93.26±9.80

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

The effect of the seasonal variations on the parasitic infections in fish is a debate issue (Soliman and Ibrahim, 2012). Elsheikha and Elshazly (2008) observed a consistent pattern of seasonal changes in prevalence of MC in *Mugil cephalus, M. capito, T. nilotica* and *T. zilli* with higher levels of MC infection in late spring and summer and the lowest level is recorded in winter. Ibrahim and Soliman (2010) reported that prevalence of *Haplorchis yokogawi* and *Pygidiopsis genata* showed no significant response to the seasonal variation while their seasonal intensities were higher in summer. On the other hand, Bello *et al.* (2000) and Simkova *et al.* (2005) claimed that an increase in the prevalence of parasitic infections is attributed to the stress of reproductive processes. Intensity of encysted MC is thus a factor that could potentially contribute to the decline in infections observed in winter, suggesting an association between higher mortality and parasitism intensity (Jensen *et al.,* 1998). The seasonal pattern, with variable amplitudes of fluctuation in different fish species in different seasons also suggests the presence of a seasonal infection-modifying factor (Elsheikha and Elshazly, 2008).

The effect of fish length on the prevalence, abundance and intensity of infection in *T. zilli* is shown in table (3). Prevalence was the highest (40.51%) in fish within length class 2 (<13), and is the lowest (33.33%) in fish within length class 3 (>13). Regarding infection with different species of MC, the highest prevalence was recorded in class 1 for *Phagicola* sp. (22.93%). In class 2, the highest prevalence was recorded for *Phagicola* sp. (16.46%).

The fishes in class 3 were mostly attacked by *Pygidiopsis* sp. (20.83%) while no infection was recorded for undifferentiated heterophyid MC. The intensity of infection (92.63±8.97) was the highest in fish within length class 1(<11). It was the lowest (51.25±3.43) in fish within length class 3 (>13) (Table 3) ($P < 0.001$). Regarding infection with different species of MC, the highest intensity of infection was recorded for *Euclinostomum* sp. in class 1 (138.09±7.94) ($P < 0.001$). In class 2, the highest intensity of infection was recorded for undifferentiated heterophyid MC (100±10.15) ($P < 0.001$). In class 3, *Phagicola* sp. caused the highest intensity of infection, and the least was by *Pygidiopsis* sp. (12.00±2.00) ($P < 0.001$). On the other hand, the highest intensity of infection with *Euclinostomum* sp. was recorded in the smallest fish. Thomas *et al.* (1981) and Hagras *et al.* (1995) found that infestation levels are the highest in medium sized fish than on smaller and larger ones. In addition, Valtonen *et al.* (1990) and Roubal (1990) found higher levels of parasitism in hosts with intermediate lengths. Furthermore, Vankara *et al.* (2011) stated that the prevalence of infection in *Mugil armatus* is low in small fish, high in medium size and decreasing in larger fish. Apparently, fish acquire the parasite in their youth phase which is then eliminated in the fish in adult phase which might be due to aging of the parasites or immunological resistance of fish (Takemoto and Pavanelli, 2000).

Table 3: Prevalence, mean abundance (±SE) and m intensity (±SE) of metacercariae infecting *T. zilli* of Lake Timsah according to host size.

Metacercariae species	Prevalence of metacercariae			Abundance			intensity		
	Class 1 (N = 157)	Class 2 (N = 79)	Class 3 (N = 24)	Class 1	Class 2	Class 3	Class 1	Class 2	Class 3
Undifferentiated Heterophyid MC	2.55% (n=4)	3.79% (n=3)	0%	1.66 ± 0.3	2.79 ±1.32	0	65.00±4.5	100.00±10.15	0
***Euclinostomum* sp.**	13.38% (n=21)	13.92% (n=11)	8.33% (n=2)	18.47±2.43	8.61±1.5	4.17± 0.6	138.09±7.94	61.81±5.46	50.00 (n=2)
***Pygidepsis* sp.**	0.64% (n=1)	5.06% (n=4)	20.83% (n=5)	1.03 ±1.03	3.29 ±0.93	2.50 ±1.09	6.67 (n=1)	65.00±6.38	12.00±2.00
***Phagicola* sp.**	22.93% (n=36)	16.46% (n=13)	8.33% (n=2)	11.22±1.23	6.15 ±1.52	7.50 ±1.3	49.17±8.32	37.69±3.22	90.00 (n=2)
***Stictodora* sp.**	6.37% (n=10)	12.66% (n=10)	12.5% (n=3)	1.35 ± 0.44	6.83 ±1.99	2.92 ±0.5	21.00±2.33	54.00±9.2	23.33±6.67
Total prevalence	36.31% (n=57)	40.51% (n=32)	33.33% (n=8)	33.6 ± 2.65	28.61 ±2.2	17.50 ±2.2	92.63±8.97	70.62±9.89	51.25±3.43

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

Results show that the prevalence with MC was higher in males (38.93%) compared to females (30.04%) (Table 4). Regarding infection with different species of MC, in males, *Phagicola* sp. was the first in respect to prevalence of infection (21.56%), and *Euclinostomum* sp. (10.74%) was the second, followed by *Pygidiopsis* sp. (5.37%), undifferentiated heterophyid MC (2.01%), and *Stictodora* sp. (0.58%). In Females, the highest prevalence was recorded for *Euclinostomum* sp. and *Phagicola* sp. (16.21%), followed by *Stictodora* sp. (9.91%), undifferentiated heterophyid MC (3.60%), and *Pygidiopsis* sp. (1.80%).

Table 4: Prevalence, mean abundance (±SE) and m intensity (±SE) of metacercariae infecting *T. zilli* of Lake Timsah according to host sex.

Metacercariae species	Prevalence of metacercariae		Abundance		Intensity	
	Male (N = 149)	Female (N = 111)	Male	Female	Male	Female
Undifferentiated Heterophyid MC	2.01% (n=3)	3.60% (n=4)	1.81±1.32	2.61±1.35	90.00±5.67	72.50±6.25
***Euclinostomum* sp.**	10.74% (n=16)	16.21% (n=18)	11.48±1.05	17.75±1.12	106.88±6.11	109.44±7.42
***Pygidepsis* sp.**	5.37% (n=8)	1.80% (n=2)	2.62±1.37	0.81±0.72	48.75±5.79	45.00±5.0
***Phagicola* sp.**	21.56% (n=32)	16.21% (n=18)	8.32±1.11	10.82±2.13	38.75±4.23	63.16±6.81
***Stictodora* sp.**	0.58% (n=12)	9.91% (n=11)	2.75±1.16	3.70±1.65	34.17±5.67	37.27±6.68
Total prevalence	38.93% (n=58)	30.04% (n=40)	26.85±2.3	35.58±2.71	70.18±4.82	98.75±6.03

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

The total mean intensity of infection was higher in females (98.75±6.03) than males (70.18±4.82) ($P < 0.001$) (Table 4). Regarding infection with different species of MC, the highest intensity of infection was recorded in males for *Euclinostomum* sp. (106.88±6.11) ($P < 0.001$). Also, the highest intensity of infection in females was recorded for *Euclinostomum* sp. (109.44±7.42) ($P < 0.001$). The intensity of infection with *Phagicola* sp. was significantly higher in female fish. Similarly, Paling (1965) found that male fish are heavily parasitized than female hosts. However, the sex differences for preferences of the infection may be attributed to the immune response of the host or to the difference in endocrine glands activities between the male and female host fish which have been suggested by many authors (Pickering and Christie, 1980). However, Poulin (1996) arrived to a conclusion that the sex difference is irrelevant and no significant differences between the prevalence and intensity of infection in female and male hosts. Effect of host sex on the levels of parasitism was studied by Thompson and Kavaliers (1994) who attributed it to the physiological and biological factors and behavioural difference between males and females. There are intrinsic biological differences between host sexes; differences that could lead one sex being more prone to parasitic infections than the other (Poulin, 1996).

On the other hand, there are studies by Luque *et al.* (1996), and Lizama *et al.* (2006) found host sex not to be a significant factor in determining the prevalence of helminth parasites in host fishes. Furthermore, Flores and Liliana (2002) found that the intensity of diplostomatid species in Lake Gutiérrez and Lake Escondido do not show significant differences between sexes and co-varied with host length. However, different levels of parasitism in females and males may result from ecological divergence between genders that could lead to different exposure to parasites (Reimchen and Nosil, 2001).

In conclusion, the present study reflects the different responses of MC infecting *T. zilli* to the environmental and biological factors which were varied according to genus of MC.

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ARABIC SUMMARY

بعض العوامل المؤثرة على الإصابة بالميتاسركاريا فى أسماك البلطى الأخضر من بحيرة التمساح ، الإسماعيلية

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هذا العمل يهدف إلى دراسة تأثير بعض العوامل البيئية والبيولوجية على انتشار الميتاسركاريا فى أسماك البلطي الأخضر من موقعين من بحيرة التمساح فى الفترة من مارس 2009 إلي فبراير 2010. تم تجميع الأسماك شهرياً وتم قياس أطوالها وتحديد نوع الجنس. أوضحت النتائج وجود 5 أنواع من الميتاسركاريا وكانت نسبة الإصابة فى المنطقة الثانية (42.4%) أعلي من المنطقة الأولى (32.59%). كانت نسبة الإصابة الأعلي خلال فصل الشتاء (79.76%) والأدنى فى الربيع (9.52%). وقد تم دراسة تأثير أطوال الأسماك علي نسبة انتشار الميتاسركاريا وأظهرت النتائج أن نسبة الإصابة كانت الأعلي في الأسماك المتوسطة فى الطول (40.51%). وتم أيضا دراسة تأثير الجنس علي وجود الميتاسركاريا و كانت نسبة الإصابة الأعلي فى الذكور (38.93%) مقارنة بالإناث (30.04%). تعكس هذه الدراسة استجابات الميتاسركاريا المختلفة للعوامل المختبرة.