PREVALENCE AND SITE PREFERENCES OF HETEROPHYID METACERCARIAE IN *TILAPIA ZILLI* FROM ISMALIA FRESH WATER CANAL, EGYPT

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Summary:
Factors affecting prevalence and site preferences of heterophyid metacercariae (MC) were investigated in this study. A total of 173 specimens of a freshwater fish, *Tilapia zilli* were collected during autumn 2008, winter 2009 and summer 2009, from Ismailia fresh water canal, Egypt. Results showed that the total prevalence of heterophyid MC was 95.37 %. The heterophyid MC consisted of *Haplorchis yokogawi* (47.4 %), *Pygidiopsis genata* (21.4 %), and *Phagicola ascolonga* (93.64 %). *H. yokogawi* MC was detected in striated muscles, *P. ascolonga* in liver and kidney while, *P. genata* was detected in muscles, liver and kidney. The aggregation indices showed that all MC were aggregated and the *P. genata* was the most highly aggregated followed by *H. yokogawi* and then *P. ascolonga*. Responses of the heterophyid MC to host sex, weight and season greatly varied according to species of MC. Different responses of interaction for heterophyid MC intensity were found by GLIM analysis and this variation dependant on the type of infection (single or mixed infections). Factors affecting site preference of heterophid MC infection were discussed and further studies in other locations are required to examine the factors affecting site preference.

KEY WORDS: *Tilapia zilli*, metacercariae, Heterophyidae, prevalence, site preference, Egypt.

Résumé : FRÉQUENCE DE MÉTACERCAIRES D'HÉTÉROPHYIDÉS CHEZ *TILAPIA ZILLI* AU NIVEAU DE DIFFÉRENTS SITES DU CANAL D'EAU DOUCE D'ISMAÏLIA EN ÉGYPTE.

Les facteurs influant sur la présence et les conditions de développement de métacercaires (MC) d'hétérophyidés dans différents sites ont été étudiés. 173 spécimens du poisson d'eau douce Tilapia zilli *ont été collectés au cours de l'automne 2008, de l'hiver et de l'été 2009, dans le canal d'eau douce d'Ismailia, en Égypte. La fréquence totale des MC de différents hétérophyidés était de 95.37 %. Ces MC étaient celles de* Haplorchis yokogawi *(47.4 %),* Pygidiopsis genata *(21.4 %) et de* Phagicola ascolonga *(93.64 %).* H. yokogawi *MC a été détectée dans les muscles striés,* P. ascolonga *dans le foie et les reins, et* P. genata *dans les muscles, le foi et les reins. La présence des différentes espèces était très variable selon le sexe et le poids de l'hôte, et selon la saison. Des variations portant sur l'intensité de l'infection par les différentes espèces ont été observées, et cela selon le type de l'infection, unique ou multiple. Les facteurs favorables à l'infection par les MC d'hétérophyidés selon les sites ont été discutés, mais des études dans d'autres sites sont nécessaires afin de mieux connaître ces facteurs.*

MOTS CLÉS : Tilapia zilli, *métacercaire, hétérophyidés, fréquence, site, Égypte.*

INTRODUCTION

Fishborne trematode infections affect the health of more than 18 million people around the world (Sohn *et al.*, 2009). These flukes provoke remarkable morbidity and cause serious damage to aquaculture in developing countries (WHO, 2005). Metacercariae (MC) of the family Heterophyidae in marine and freshwater fishes are non specific in their choice of definitive hosts, and therefore, cause infections in domestic animals and human consuming raw or incompletely cooked fish (Dzikowski *et al.*, 2003).

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, while factors affecting occurrence and population dynamics of heterophyid metacercariae (MC) in fish are far less characterized (Sithithaworn *et al.*, 1997; Massoud *et al.*, 2007; Elsheikha & 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).

Parasitologists have had a long-standing interest in understanding and characterizing the specific sites that parasites occupy within individual hosts (Sukhdeo & Sukhdeo, 2002, 2004). This interest in parasite site selection stems from its pivotal role in many aspects of host-parasite interactions, including parasite transmission dynamics, parasite-induced host pathology, and parasite taxonomy and phylogeny (Williams, 1966; Sukhdeo & Sukhdeo, 1994). Unfortunately, studies on the factors

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possibly affecting the site preference of heterophyid MC are scarce. Ciurea (1924) found a certain preference to heterophyid MC site of encystment in the fish host, depending upon the species to which they belong. Therefore, more studies are required to elucidating the proximate and ultimate causes of extreme site specificity, particularly for those parasites that contribute to human and livestock.

About 30 Heterophyid metacercaria species of nine genera, i.e., *Metagonimus, Heterophyes, Heterophyopsis, Stellantchasmus, Centrocestus, Pygidiopsis, Procerovum, Haplorchis, and Stictodora, Haplorchis, phagicola* and *Pygidiopsis* are known to be human-infecting heterophyid flukes (Chai & Lee; 2002; Kim *et al*., 2006). In terms of sources of infection, several kinds of brackish and fresh water fish, such as bolti (*Oreochromis* sp.), mullet (*Mugil* sp) and *Tilapia* sp. have been identified in Egypt (Khalil, 1933; Martin, 1959; Taraschewski, 985). *Tilapia zilli* is common in Egypt and is sometimes heavily infected with the metacercariae of heterophyid flukes, and are popularly eaten undercooked by residents.

Despite a huge literature on trematode metacercariae ecology and epidemiology, little is known about them in their natural hosts in Egypt (Elsheikha & Elshazly, 2008). Moreover, the interaction between biotic and abiotic factors and the possible influence on coexistence and site preferences were scarcely studied. Therefore, the present study aimed to investigate the infection status of heterophyid fluke metacercariae in *Tilapia zilli* and study the factors involved in the occurrence and site preferences of heterophyid MC.

MATERIALS AND METHODS

A total of 173 specimens of *Tilapia zilli* were caught from Ismailia fresh water canal, Ismailia, Egypt (30°35'27.56" N, 32°17'54.91" E), during Autumn 2008, Winter 2009 and summer 2009. The collected fish were placed in ice-boxes, and then transported to the laboratory. Host sex was determined according to Guerrero and Shelton (1974). 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. Weights of the collected fish were measured and ranged from 1.9 to 57 g. Fish weight classified into three weight classes (class 1: 1-5 g; class 2: > 5-12 g; class 3: > 12 g). The structure of the sampled host population by the season of capture and host sex is shown in Table I.

Initially, each fish was screened for the presence of metacercariae (MC) by compression method in which snipes were taken from different body regions muscles and visceral organs such as liver and kidney. Each piece was compressed between two microscopic glass slides and examined for the presence of MC. Metacercariae identification was based on those dissected directly from tissues or from squash preparation. They were separately collected by the general feature and were tentatively identified to species level based on the morphological details, their dimensions and being either singly or in groups (Sohn *et al*., 2009; Elsheikha & Elshazly, 2008). The intensity of MC infection was done by digestion method. The digestion was performed using 1 % pepsin digestive solution in normal saline (1 ml conc. hydrochloric acid, 1 g pepsin, 99 ml 0.85 % sodium chloride solution for 1.3 hr at 37°) (Nithikathkul & Wongsawad, 2008). The digested material was then rinsed with 0.85 % sodium chloride solution and examine for MC. The number of metacercariae was calculated per gram of tissue.

Season	Host sex		Total
	Male	Female	
Summer	77	32	109
Autumn	32	6	38
Winter	18	8	26
Total	127	46	173

Table I. – Structure of the sampled host population of *T. zilli* by season of capture and host sex.

The prevalence and mean intensity of metacercarial infection were estimated according to Bush *et al*. (1997). The degree of aggression of different species of parasites was calculated by the Index of Discrepancy (D) as described by Poulin (1993) (a value of 0 indicates an even distribution of accounts across all hosts and a value of 1 indicate all parasites aggregated in a single host). Factors affecting the metacercarial intensity were analyzed by GLIM (General linear models) using a model with the negative binomial distribution error. To satisfy the assumption of statistical analysis of GLIM, all the data were normalized by log (x+1) transformation to achieve homoscedasiticity or linearity Prevalence is the proportion of infected hosts, and mean intensity is the mean number of parasites per infected host. Mann-Whitney (*U* test) was used to test the differences in intensity per host sex whereas Kruskal-Wallis test was used to test the difference in encysted MC intensity per season and host weight classes. The difference in encysted MC prevalence was tested using Chi-square test (χ^2). Correlations between intensity of infection and weight classes were examined using the non-parametric, Spearman's rank correlation coefficients (r_s). Differences between the prevalence and intensities of infection relative to season, and host sex were assessed using Chi-squire. In all cases, significant difference was set at $P \leq 0.05$. All the statistical tests were performed by using the software packages SPSS 15.0 (USA).

RESULTS

Out of 173 examined *Tilapia zilli*, 165 were infected with one or more species of encysted heterophyid metacercariae with overall prevalence rate 95.37 %. Three species of encysted MC were detected in this survey; they were *Haplorchis yokogawi, Pygidiopsis genata and Phagicola ascolonga*. *H. yokogawi* was detected in striated muscles of examined fish while *P. ascolonga* was in liver and kidney. *P. genata* was detected in muscles, liver and kidney. The overall prevalence of encysted metacercariae *H. yokogawi, P. genata* and *P. ascolonga* were 47.4 % (82/173), 21.4 % (37/137) and 93.64 % (162/173), respectively. The aggregation index showed that all recorded MC were aggregated in *T. zilli*. The *P. genata* was the most highly aggregated (0.82) followed by *H. yokogawi* (0.60) and then *P. ascolonga* (0.12).

The prevalence of encysted metacercariae *H. yokogawi* differed significantly by host sex (Table II; χ^2 = 7.19, P = 0.007). Male had higher infection prevalence 53.5 (68/126) than that in female fish (Table II). The intensity of infection in *P. ascolonga* differed significantly by host sex (Table II: U = 2168.50, P = 0.01). The mean infection intensity of *P. ascolonga* was higher in females (242.85 ± 74.69) than that in males (74.14 ± 10.25) (Table II).

The results showed that infection prevalence differed significantly by host weight classes in *P. ascolonga* (Table III; χ^2 = 7.65, P < 0.02). Significant differences were found in mean intensity of *P. ascolonga* by host weight classes

Encysted metacercariae	Host sex	Parameters	Muscle	Liver	Kidney	Total
H. yokogawi	Combined	Mean intensity ± SE (Range)	56.11 ± 7.74 (4-370)	0	0	56.11 ± 7.74 (4-370)
		Prevalence (%) a/b	47.4 (82/173)	0	0	47.4 % (82/173)
	Male	Mean intensity ± SE (Range)	45. 09 ± 6.75 (4-310)	0	0	46. 42 ± 6.68 (4-310)
		Prevalence (%) a/b	53.5 (68/126)	0	0	53.5 (68/126)
	Female	Mean intensity ± SE (Range)	109.64 ± 27.94 (4-370)	0	0	109.64 ± 27.94 (4-370)
		Prevalence (%) a/b	30.4 % (14/46)	0	0	30.4 % (14/46)
P. ascolonga	Combined	Mean intensity ± SE (Range)	0	172.45 ± 35.92 (10-2200)	25.11 ± 3.60 (1-480)	117.88 ± 21.44 (1-2246)
		Prevalence (%) a/b	0	51.5 % (89/173)	71.09 % (162)-	93.64 % (162/173)
	Male	Mean intensity ± SE (Range)	0	110.37 ± 16.03 (20-640)	21.70 ± 2.74 (1-124)	74.14 ± 10.25 (1-676)
		Prevalence (%) a/b	0	44.9 % (57/126)	95.3 % (120)	95.3 % (120/126)
	Female	Mean intensity ± SE (Range)	0	283.03 ± 93.53 (10-2200)	34.83 ± 11.44 (1-480)	242.85 ± 74.69 (1-2246)
		Prevalence (%) a/b	0	69.6 % (32/46)	8.7 % (42)	91.3 % (42/46)
P. genata	Combined	Mean intensity ± SE (Range)	13.05 ± 2.04 (2-35)	21.62 ± 4.30 (2-67)	18.50 ± 4.86 (2-48)	17.88 ± 2.89 (2-83)
		Prevalence (%) a/b	12.1 % (21/173)	9.8 % (17/173)	5.8 % (10/126)	21.4 % (37/137)
	Male	Mean intensity ± SE (Range)	10.61 ± 1.72 (2-20)	19.81 ± 5.77 (2-67)	16 ± 5.07 (2-35)	14.72 ± 2.77 (2-67)
		Prevalence (%) a/b	11.8 % (15/126)	9.5 % (12/126)	5.5 % (7/126)	20.5 % (26/126)
	Female	Mean intensity ± SE (Range)	19.40 ± 5.14 (3-35)	25.60 ± 5.73 (3-35)	24.33 ± 12.25 (2-35)	25.09 ± 6.80 (3-83)
		Prevalence (%) a/b	13.04 % (6/46)	10.9 % (5/46)	6.5 % (3/46)	23.9 % (11/46)

Table II. – Intensity (mean ± SE) with the range of infection and prevalence of heterophyid encysted metacercariae (MC) in *T. zilli* per host sex and organ.

(χ^2 = 21.05, P < 0.001). A higher prevalence and intensity were recorded in higher host weight class (Table III). Positive associations were found between weight classes and intensity of *P. ascolonga* (r_s = 0.33, P < 0.001).

The infection prevalence of MC differed significantly by season (χ^2 = 1475, P = 0.001). Summer showed the highest infection prevalence (98.16 %; 107/109) when compared with that in autumn (97.63 %; 37/38) and winter (80.76 %; 21/26). There was a significant difference in infection prevalence among seasons in *P. ascolonga* (χ^2 = 16, P < 0.001). Infection prevalence was higher in summer (98.16%: 107/109), when compared with that in other seasons (Table IV). A significant difference was found among seasons in mean intensity of *P. ascolonga* MC (χ^2 = 33.60, P < 0.001). The highest mean intensity was in summer (154.11 ± 31.39 followed by autumn (61.03 ± 17.30) and then winter (Table IV: 23.55 ± 8.44).

The examination of organ distribution of heterophyid MC indicated that kidney and liver were the most frequently infected organs. Significant differences were found in infection prevalence among examined organs (Table II). Significant difference was found in intensity of *P. asolonga* between liver and kidney (U = 7760.500, P = 0.001). Examination of striated muscle of male and female fish showed that there was a significant difference in infection prevalence in *H. yokogawi* infection (Table II; χ^2 = 7.19, P = 0.007). Male fish had higher infection prevalence in muscle 53.5 % (68/126) than that in female ones (Table II). *P. ascolonga* indicated that kidney (94.79 %: 164/173) was the most frequently infected compared with liver (50.89 %: 87/173) of infected fish. Prevalence of *P. ascolonga* in liver of fish differed significantly between host sexes (Table II; χ^2 = 8.19, P = 0.004). Prevalence of *P. ascolonga* in liver of female *T. zilli* was higher (69.6 %) than that in male (Table II; 44.9 %). Significant difference was found in infection intensity of *P. ascolonga* between male and female (U = 2042.50, P = 0.001). The examination of organ distribution of *P. genata* indicated that muscle was the most frequently infected organ followed by liver and kidney (Table II).

GLIM analysis was performed including seasons, host sex and weight. Responses of heterophyid MC intensity

Encysted metacercariae		Weight class 1	Weight class 2	Weight class 3	Total	P value
H. yokogawi	Prevalence a/b (%)	20/43 (46.51)	39/78 (50)	23/52(44.23)	82/137 (47.39)	0.81
	Mean intensity ± SE (Range)	43.70 ± 7.02 (10-150)	74.31 ± 14.84 (4-370)	39.98 ± 7.68 (4-150)	56.11 ± 7.74 (4-370)	0.70
P. ascolonga	Prevalence a/b (%)	37/43 (86.05)	73/78 (93.58)	52/52 (100)	162/173 (93.64)	0.02
	Mean intensity ± SE (Range)	38.78 ± 9.01 (1-205)	125.36 ± 31.13 (1-2121)	163.67 ± 49.30 (1-2246)	117.88 ± 21.44 (1-2246)	< 0.001
P. genata	Prevalence a/b (%)	12/43 (27.91)	16/78 (20.51)	8/37 (15.38)	36/173 (20.81)	0.32
	Mean intensity ± SE (Range)	8.91 ± 2.47 (2-30)	21 ± 4.70 (2-83)	25.12 ± 7.17 (3-67)	17.88 ± 2.89 (2-83)	0.01

a/b: no. of infected/no. of examined.
Significant level at P < 0.05

Table III. – Prevalence (%) and mean intensity (± SE) of heterophyid encysted metacercariae (MC) in *T. zilli* per host weight class.

Encysted metacercariae		Summer	Autumn	Winter	Total	P value
H. yokogawi	Prevalence a/b (%)	47/109 (43.12)	21/38 (55.26)	14/26 (53.85)	82/173 (79.19)	0.33
	Mean intensity ± SE (Range)	70.82 ± 11.37 (6-370)	37.55 ± 14.28 (4-31)	34.57 ± 6.97 (4-75)	56.11 ± 7.74 (4-370)	0.01
P. ascolonga	Prevalence a/b (%)	107/109 (98.16)	35/38 (92.10)	20/26 (76.92)	162/173 (93.64)	< 0.001
	Mean ± SE (Range)	154.11 ± 31.39 (1-2246)	61.03 ± 17.30 (2-459)	23.55 ± 8.44 (1-104)	117.88 ± 21.44 (1-2246)	< 0.001
P. genata	Prevalence a/b (%)	24/109 (22.02)	5/38 (13.16)	7/26 (26.92)	36/173 (79.19)	0.39
	Mean ± SE (Range)	21.58 ± 3.86 (2-83)	20 ± 5.13 (2-30)	3.71 ± 0.77 (2-8)	17.89 ± 2.89 (2-83)	0.007

a/b: no. of infected/no. of examined.
Significant level at P < 0.05

Table IV. – Seasonal prevalence and mean intensity of heterophyid encysted metacercariae (MC) in *T. zilli*.

Organs	Type of infection	MC	Factors	Sum of squares	df	Mean square	F	Sig.
Muscles	Single	*H. yokogawi*	wtclass	3150.799	2	1575.400	2.718	0.070
			season * sex	1737.053	1	1737.053	2.997	0.086
			season * wtclass	5948.468	2	2974.234	5.132	0.007
			sex * wtclass	3473.333	2	1736.667	2.996	0.053
			season * sex * wtclass	2770.796	2	1385.398	2.390	0.095
		P. genata			No significant effects			
	Mixed	*H. yokogawi*	season	30254.047	2	15127.024	3.648	0.058
			sex	48050.000	1	48050.000	11.588	0.005
			wtclass	42828.839	2	21414.420	5.164	0.024
			season * wtclass	23960.000	2	11980.000	2.889	0.095
		P. genata	season	218.926	2	109.463	3.390	0.068
			sex	296.056	1	296.056	9.168	0.011
Liver	Single	*P. ascolonga*	wtclass	191601.211	2	95800.606	2.700	0.071
			sex * wtclass	607779.879	2	303889.940	8.563	0.000
		P. genata			No significant effects			
	Mixed	*P. ascolonga*			No significant effects			
		P. genata			No significant effects			
Kidney	Single	*P. ascolonga*	season	6816.061	2	3408.030	6.165	0.003
		P. genata			No significant effects			
	Mixed	*P. ascolonga*	season	78450.286	1	78450.286	52.241	0.002
			sex	13382.086	1	13382.086	8.911	0.041
			wtclass	66379.114	1	66379.114	44.203	0.003
			season * sex	42130.286	1	42130.286	28.055	0.006
			sex * wtclass	58400.205	1	58400.205	38.890	0.003
		P. genata			No significant effects			

Table V. – Results of the General Linear Modelling (GLIM) analyses of interaction between season, host sex and weight and the intensity of heterophyid encysted metacercariae (MC).

to the studied factors varied according to the type of infection (single or mixed infections). Season, host weight and host sex did not affect the intensity with *H. yokogawi* in single infection while, significant affects were found when there was a mixed infection with *P. genata* (Table V). Season was the only limiting factor of infection intensity with *P. ascolonga* in kidney in single infection. In mixed infection with *P. ascolonga* and *P. genata*, season, host sex and weight class significantly affected the intensity. Also, there was interaction between Season and host sex and host sex and weight class (Table V). *P. genata* did not respond to the factors in case of single or combined infections. Interestingly, infections in liver did not show significant responses to the studied factors in single or mixed infection. Only host sex interacted with host weight significantly affected the intensity of infection with *P. ascolonga* in liver in case of single infection.

From the interaction data, season was the only limiting factor of infection intensity with *P. ascolonga* in kidney in single infection. In mixed infection with *P. ascolonga* and *P. genata*, season, host sex and weight class significantly affected the intensity. Also, there was interaction between Season and host sex and host sex and weight class (Table V). *P. genata* did not respond to the factors in case of single or combined infections. Interestingly, infections in liver did not show significant responses to the studied factor in single or mixed infection. Only host sex interacted with host weight significantly affected the intensity of infection with *P. ascolonga* in liver in case of single infection.

DISCUSSION

In this study, *Haplorchis yokogawi*, *Pygidiopsis genata* and *Phagicola ascolonga* metacercariae that recorded are commonly found in the fresh water fish of Egypt, *Oreochromis niloticus* and *T. zillii* (Elsheikha & Elshazly, 2008; Taher 2009; Abdallah *et al.*, 2009). Through this study, the prevalence of heterophyid MC was 95.37 %. This finding was higher than that reported by Abd-El-Rahman (2005) 45 %, Abdallah *et al.* (2009) 91.7 % and Taher (2009) 78.25 %.

Results showed that *P. ascolonga* was the most prevalent (93.64 %) followed by *H. yokogawi* (47.4 %) and *P. genata* (21.4 %). The prevalence of *H. yokogawi* was high compared with Taher (2009), 26%. 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.

The examination of organ distribution of heterophyid MC indicated that *H. yokogawi* MC found in striated muscles, *P. ascolonga* in liver and kidney while, *P. genata* was detected in muscles, liver and kidney. In contrast, Elsheikha & Elshazly (2008) detected *H. yokogawi* and *P. genata* MC in striated muscles only, while *P. ascolonga* was found in mesenteries adjacent to the small intestine and attached to the visceral peritoneum, liver and gall bladder. These findings reflect site preference for each species, which in turn may be related to the difference in host species and location. Similarly, Kino *et al.* (2006) observed site preference in *Metagonimus yokogawai* and *Metagonimus miyatai* metacercariae and may explain the characteristic site preference and possible existence of genetic variation of *M. Yokogawai*. Site preference of infection may be attributed to many factors, including host species and condition, geographical distribution and genetic variation of MC (Kawanaka *et al.*, 2002; Kino *et al.*, 2006). Site preference of infection may be related to co-infection of heterophid MC in single organ as represented in this study. A single host may harbour more than one type of metacercariae.

Infection intensity was higher in females when compared with that in males in the three species of MC. The sex differences in infection may be attributed to the immune response of the host due to the difference in endocrine glands activities between the male and female host fishes which have been suggested by many authors (Donaldson & Fagerlund 1970; Poulin 1996; Gbankoto *et al.*, 2001). The higher intensity in female ones in the three species of MC may be related to investment in reproduction of females is more costly than that in males, thus female are more susceptible to parasite infection in periods of investment in gonad development (Simkova *et al.*, 2005). However, Poulin (1996) concluded that the sex difference is irrelevant and no significant differences between the prevalence and intensity of infection in female and male hosts.

The results showed that a higher prevalence and intensity of *P. ascolonga* were recorded in the higher host weight class. Similarly, Taher (2009) showed that the infection rate of MC in *O. niloticus* was higher in larger fish. In contrast, Chappell (1969) also revealed that the infestation level of parasites generally attained a negative relationship with host size of fish.

Prevalence of *H. yokogawi* and *P. genata* showed no significant response to the seasonal variation while their seasonal intensities were higher in summer. *P. ascolonga* prevalence and intensity showed significant response to seasonal variation being higher in summer. Seasonal-dependent variations in the occurrence of heterophyid MC in particular were previously reported by being the highest in summer (Abou-Zakham *et al.*, 1990, Elsheikha & Elshazly, 2008). The reason for this phenomenon was attributed to temperature-dependent release of cercariae (Makhlouf *et al.*, 1987; Elsheikha & Elshazly 2008). Seasonal variations in prevalence and intensity of heterophyid MC infection has been previously documented and explained by the temperature-dependent release of cercariae (Makhlouf *et al.*, 1987; Elsheikha & Elshazly, 2008). Moreover, the difference in prevalence and intensity of MC between seasons is believed to be due to a difference in the time of exposure to parasites. Indeed, release of trematode cercariae from the snail host and successful transmission to the fish host is highly temperature-dependent (Oshima & Nishi, 1963). On the other hand, the decreased level of MC during the cold season may be explained by death of the cercariae/metacercariae. Additional factors including water conditions and first intermediate host availability may cause parasite prevalence and intensity to vary in the manner we observed (Taher, 2009; Elsheikha & Elshazly, 2008). However, a complete understanding of the association between snail vector abundance, and prevalence and intensity of heterophyid MC requires knowing the snail vector's identity, its seasonal abundance, and the prevalence of heterophyids in the snail vector. We do not currently have all those data.

It is concluded that heterophyid MC are highly prevalent in *T. zilli* caught from Ismailia canal, Egypt. Responses of heterophyid MC to host sex, weight and season in prevalence and intensity varied according to species of heterophyid MC. Further studies in other locations are required to examine the factors affecting site preference.

REFERENCES

ABDALLAH K.F., HAMADTO H.H., EL-HAYAWAN I.A., DAWOUD H.A., NEGM-ELDIN M. & AHMED WEL-A. Metacercariae recovered from fresh-water fishes in the vicinity of Qualkyobia governorate, Egypt. *Journal of Egyptian society of parasitology,* 2009, *39* (2), 467-477.

ABD-EL-RAHMAN, A.M.M. Studies on prevailing parasitic diseases among some fresh water fishes caused by Digenetic trematodes. *SCVMJ*, 2005, *8* (1), 13-24.

ABOU-ZAKHAM A., EL-SHAZLEY A., EL-GANAYNI F., ROMIA S.A., ABOU-SHADY A.F., EL-KHOLY E.I. *et al.* Seasonal variation and incidence of *Stictodora tridactyla* in fish from lake Manzala. *Journal of Egyptian society of parasitology,* 1990, *20* (1), 117-121.

BUSH A.O., LAFFERTY K.D., LOTZ J.M. & SHOSTAK A.W. Parasitology meets ecology on its own terms: Margolis *et al.*, revisited. *Journal of Parasitology,* 1997, *83*, 575-583.

Chai J.Y. & Lee S.H. Food-borne intestinal trematode infections in the Republic of Korea. *Parasitology International*, 2002, *51* (2), 129-154.

Chappell L.H. The Parasites of the Three-spined Stickleback *Gasterosteus aculeatus* L. from a Yorkshire Pond. II. Variation of the parasite fauna with sex and size of fish. *Journal of fish Biology*, 1969, *1*, 339-347.

Ciurea I. Heterophyides de la faune parasitaire de Roumanie. *Parasitology*, 1924, *16*, 1-21.

Donaldson E.M. & Fagerlund U.H. Effect of sexual maturation and gonadectomy at sexual maturity on cortisol secretion rate in sockeye salmon (*Oncorhynchus nerka*). *Journal of fish Research*, 1970, *27*, 2287-2296.

Dzikowski R., Diamant A. & Paperna I. Trematode metacercariae of fishes as sentinels for a changing limnological environment. *Disease of Aquatic Organisms*, 2003, *55*, 145-150.

Elsheikha H.M. & Elshazly A.M. Host-dependent variations in the seasonal prevalence and intensity of heterophyid encysted metacercariae (Digenea: Heterophyidea) in brackish water fish in Egypt. *Veterinary Parasitology*, 2008a, *153* (1-2), 65-72.

Gbankoto A., Pampoulie C., Marques A. & Sakiti G.N. Occurrence of myxosporean parasites in the gills of two *Tilapia* species from Lake Nokoué (Bénin, West Africa): effect of host size and sex, and seasonal patterns of infection. *Disease of Aquatic Organisms*, 2001, *44*, 217.

Guerrero R.D. & Shelton W.L. An aceto-carmine squash technique for sexing juvenile fishes. *Fish-Culture*, 1974, *36*, 56.

Kawanaka M., Sugiyama H., Sakamoto K., Morishima Y., Masu H., Murata I. *et al*. An epidemiological survey of *Metagonimus yokogawai* in Kasumigaura-Kitaura district. *Clinical Parasitology*, 2002, *13*, 132-135.

Khalil M. The history of human trematode parasite Heterophyes in Egypt. *Lancet*, 1933, *2*, 225-237.

Kim D.G., Kim T., Cho S., Song H. & Sohn W. Heterophyid metacercarial infections in brackish water fishes from Jinju-Man (Bay), Kyongsangnam-do, Korea. *Korean Journal of Parasitology*, 2006, *44* (1), 7-13.

Kino H., Suzuki T., Oishi H., Suzuki S., Yamagiwa S. & Ishiguro M. Geographical distribution of *Metagonimus yokogawai* and *M. miyatai* in Shizuoka Prefecture, Japan, and their site preferences in the sweetfish, *Plecoglossus altivelis*, and hamsters. *Parasitology International*, 2006, *55*, 201-206.

Makhlouf L.M., Abou Zakham A., El-Kholy E.I., Abou-Shady A.F., Abdel-Magied S. & El-Shazely A. Heterophyids of some fresh water fish from Mansoura, Egypt. *Journal of Egyptian society of parasitology*, 1987, *17*, 573-576.

Martin W.E. Egyptian heterophyid trernatodes. *Trans. Am. Microsc. Soc.*, 1959, *78*, 172-181.

Massoud A.M., El-Shazly A.M. & Morsy T.A. Mirazid (*Commiphora molmol*) in treatment of human heterophyiasis. *Journal of Egyptian society of parasitology*, 2007, *37* (2), 395-410.

Nithikathkul C. & Wongsawad C. Prevalence of *Haplorchis taichui* and *Haplorchoides* sp. Metacercariae in freshwater fish from water reservoirs, Chiang Mai, Thailand. *Korean journal of Parasitology*, 2008, *46* (2), 109-112.

OshimaY. & Nishi S. Ayu fish transplantation from Lake Biwa and the epidemiology of *Metagonimus yokogawi* infection in Japan. *Bulletin of Institute of public health of Tokyo*, 1963, *12*, 29-33.

Poulin R. Sexual inequalities in Helminth infections. *The American naturalist*, 1996, *147* (2), 287-295.

Poulin R. Comparison of three estimators of species richness in parasite component communities. *Journal of Parasitology*, 1998, *84* (3), 485-490.

Simkova A., Jarkovsky J., Koubkova B., Barus V. & Prokes M. Associations between fish reproductive cycle and the dynamics of metazoan parasite infection. *Parasitology Research*, 2005, *95*, 65-72.

Sithithaworn P., Pipitgool V., Srisawangwong T., Elkins DB. & Haswell-Elkins M.R. Seasonal variation of *Opisthorchis viverrini* infection in cyprinoid fish in north-east Thailand: implications for parasite control and food safety. *Bulletin of World Health Organization*, 1997, *75* (2), 125-131.

Sohn W., Eom K.S., Min D., Rim H., Hoang E., Yang Y. & Li X. Fishborne Trematode metacercariae in freshwater fish from Guangxi Zhuang autonomous region, China. *Korean journal of Parasitology*, 2009, *47* (3), 249-257.

Sukhdeo M.V. & Sukhdeo S.C. Fixed behaviours and migration in parasitic flatworms. *International Journal of Parasitology*, 2002, *32* (3), 329-342.

Sukhdeo M.V. & Sukhdeo S.C. Optimal habitat selection by helminthes within the host environment. *Parasitology*, 1994, *109*, 41-55.

Sukhdeo M.V. & Sukhdeo S.C. Trematode behaviours and the perceptual worlds of parasites. *Canadian Journal of Zoology*, 2004, *82*, 292-315.

Taher G.A. Some studies on metacercarial infection in *Oreochromis niloticus* in Assiut governorate and their role in transmission of some trematodes to dogs. *Assuit University Bulletin of Environmental Research*, 2009, *12* (1), 63-79.

Taraschewski H. Investigations on the prevalence of Heterophyes species in twelve populations of the first intermediate host in Egypt and Sudan. *Journal of Tropical Medicine and Hygiene*, 1985, *88*, 265-271.

WHO. Control of foodborne trematode infections. WHO Technical Report No. 849, 1995, pp 1-157.

Williams M.O. Studies on the morphology and life cycle of *Diplostomum gasterostei* (Strigeida: Trematoda). *Parasitology*, 1966, *56*, 693-706

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