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MIRACIDIOPHAGIC AND CERCARIOPHAGIC ACTIVITY OF THE FISH *GAMBUSIA AFFINIS* AND *OREOCHROMIS NILOTICUS* AND THEIR EFFECT ON THE INFECTION OF *BIOMPHALARIA ALEXANDRINA* BY SCHISTOSOMA MANSONI MIRACIDIA

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ABSTRACT

Miracidiophagic and cercariophagic activity of two fish species namely *Gambusia affinis* and *Oreochromis niloticus* and their possible role in controlling the infection of *Biomphalaria alexandrina* with *Schistosoma mansoni* mracidia were studied. The results showed that the infection rates of *B. alexandrina* exposed for about 3000 miracidia in presence of each fish species were significantly lower than the controls. There was an inverse relation between the number of *O. niloticus* and the infection rate of *B. alexandrina*. Conversily, in case of *G. affinis*, the rate of infection of *B. alexandrina* was increased by increasing the number of fish added to the same volume of water. The miracidiophagic activity of *O. niloticus* indicated that the average percentage of miracidia consumed by fish after 60 minutes exposure were 71.05 % and 78.2 % when fish were exposed to approximately 1500 and 3000 miracidia respectively. The cercariophagic activity of *G. affinis* and *O. niloticus* showed that the reduction in the number of cercariae offered to each fish species were significantly higher than the controls. The consumption of cercariae by each fish species was greatly influenced by cercariae concentration; the higher the concentration, the more cercariae consumed by the fish.

INTRODUCTION

The miracidiophagic and cercariophagic activity of different fish was clearly demonstrated in both laboratory and field studies.

Several species of tropical fishes were reported to actively ingest cercariae of *S. mansoni* (Nasir, 1979). Moreover, Ismail (1990) has shown that *Gambusia affinis* was a good potential fish species which preys on miracidia. Other studies on the miracidioiphagic and cercariophagic activities of a similar cyprinodontid species, the guppy, *Lebistes reticulatus* were carried out by several authors. Oliver-Gonzalez (1946) observed that guppies fed readily on cercaria shed by the snails. In a laboratory experiment carried out by Rowan (1958); four guppies in one liter of water consumed over 7000 *Schistosoma mansoni* cercariae in less than 3 hours. In another study, when guppies were introduced into spring water containing about 1000 cercariae per fish, a dramatic reduction in the number of cercariae was observed within 60 minutes (Pellegrino *et al.,* 1966). Similarly, Knight *et al.* (1970) studied the cercariophagic activity of the guppy, *L. reticulatus* by feeding them cercariae of *S. mansoni* labeled with radioselenium. Separate counts of radioactivity of the intestine of fish compared with the head and body proper were relatively high, indicating ingestion of cercariae. On the other hand, Muraleedharan *et al.* (1975) stated that guppy was found to devour an average of 1077 *S. mansoni* cercariae (out of 1147) within 60 minutes. About half the cercariae were consumed within the first 15 to 30 minutes, indicating that consumption was related to cercarial concentration. They added that miracidia were devoured by a single guppy in 60 minutes. Bunng *et al.* (1977) observed that when five guppies were introduced into 120 ml of water containing 500 and 1200 miracidia on two corresponding tests, a dramatic reduction in number of miracidia was observed. Also, the predation of cercariae by the fish *Poecilia reticulate* was investigated by Jordan (1985) who found that when fish were introduced into beakers containing cercariae, there were a significant reduction in number of cercariae was obtained, compared to control experiment. Heavily gravid females of *P. reticulate*, which apparently find cercariae unattractive, served as controls; where juvenile and adults, both male and females ingested many cercariae of *S. mansoni,* indicating that cercariophagic activity is not sex or age-related (Bay and Self, 1972).

Upatham (1970, 1972) reported that fish and tadpoles as well as other snails were shown to interfere with the infection of *Biomphalaria glabrata* by *S. mansoni* miracidia. Upatham and Sturock (1973) studied the effect of the fish *P. relticulata* on the infection of *B. glabrata* by *S. mansoni* miracidia. They found that this fish was used as a decoy, diminishing the target snail infection rate. In a laboratory experiment, Bunng *et al.* (1977) found that infection rates of *B. glabrata* exposed to approximately 3000 miracidia in presence of the guppy, *L. reticulatus* were greatly reduced .

With this back ground, the present work was designed to study the role of both fish species *G. affinis* and *O. niloticus* in limiting the infection of *B. alexandrina* with *S. mansoni* miracidia under laboratory conditions. Furthermore, laboratory experiments were designed to evaluate the miracidiophagic and cercariophagic activity of each of the two fish species.

MATERIAL AND METHODS

The fish *G. affinis* were collected from their natural habitats at Giza Governorate by a small Seine net, 3 X 1.2 m, made of 1 nm mesh size. While *O. niloticus* fish were brought from fish hatchery at Al-Abbassa, Sharkiah Governorate. The captured fishes were transported to the laboratory in plastic tanks containing dechlorinated water and aerated by oxygen. Fishes were maintained in laboratory aquaria for at least two weeks. Miracidia and cercariae were obtained from Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute. Both miracidia and cercariae used in the experiments were freshly emerging (within one hour) and showed active swimming.

The experiments were conducted simultaneously on the different days for each of the two fish species as follows:

For each fish species, two identical tests were carried out except for the difference in the number of fish in each test. *G. affinis* (1.8-2.8 cm in standard length) and *O. niloticus* (2.5-3.5 cm in standard length) were counted and placed in plastic aquaria (15 X 15X 8 cm) containing 1.5 liters of dechlorinated tap water. Twenty laboratory-bred *B. alexandrina* (6-8 cm in diameter) were put in the same aquaria.

Test A : Ten fish and 20 snails in each of the three plastic aquaria.
Test B: Twenty fish (in case of *G. affinis*) and 15 fish (in case of *O. niloticus*) and 20 snails in the same number of experimental aquaria.

Control groups were also containing the same number of snails in each aquarium without fish. These aquaria were left in quiet place exposed to indirect natural light for 16 hours without any given food. Then, a suspension of an estimated 2000 miracidia was introduced into each aquarium. The exposure time was 4 hours and the fish of each species were removed at the end of the experiment. The snails were maintained in the snails room and observed for comparison of infection rates 28 days after exposure. In a previous study, Ismail (1990) found that *G. affinis* feeds on miracidia. The miracidiophagic activity of the fish was greatly influenced by miracidial concentration. Thus, in the present study, another experiment was designed to test the miracidiophagic activity of only *O. niloticus* fish. The experiment was conducted in the laboratory on two different days as follows: Five young *O. niloticus* (size: 1.8-3 cm in standard length) were introduced into a beaker containing 400 ml of dechlorinated tap water, 2 hours prior to adding about 1500 miracidia. Four tests and one control without fish, were conducted at one time. All the groups were kept in quite, indirect sunlight during exposure. After one hour the fish were removed and miracidial counts in three samples of 1 ml were made. The experiment was repeated on another day with fish of approximately the same size while the number of miracidia were increased to 3000.

The cercariophagic activity of the fish *G. affinis* and *O. niloticus* was studied under laboratory conditions. For each fish species, five young fish (size 1.8-2.5 cm for *G. affinis* and 2-3 cm for *O. niloticus* in standard length) were introduced into a beaker containing 400 ml of dechlorinated water with about 3000 *S. mansoni* cercariae. Cercarial counts were performed after 60 minutes, in three samples of 1 ml. The experiment was repeated on another day for each fish species of approximately the same size while the number of cercaria was increased to 12000 (in case of *G. affinis*) and to 6000 (in case of *O. niloticus*).

RESULTS AND DISCUSSION

The results obtained in the present study suggest that the two fish species *G. affinis* and *O. niloticus* are probable predators of *S. mansoni* miracida, cercariae and may play an important role as a limiting or interfering factor in the infection of *B. alexandrina* snails by *S. mansoni* miracidia. Present investigation showed that the reduction in the infection rates of *B. alexandrina* exposed to miracidia of *S. mansoni* in presence of the fish *G. affinis* (18.18 % in the test A and 25.06 % in test B) were significantly ($p < 0.001$) lower than the controls (78.57 % and 75% in test A and B, respectively), (Table 1).

Moreover, the reduction in infection rate of *B. alexandrina* exposed to miracidia in presence of the fish *O. niloticus* (33.57% in test C and 22.16% in test D) was significantly ($p< 0.001$) lower than the controls (66.67% and 63.16%, respectively) (Table 2). Thus, there is an inverse relation between the number of *O. niloticus* and the infection rate of *B. alexandrina*. In contrast, in case of *G. affinis* it was found that the rate of infection of *B. alexandrina* by *S. mansoni* miracidia was increased with increasing the number of fish added to the constant volume of water. These findings agree with those of Bunnag *et al.* (1977) on the miracidiophagic activity of a similar cyprinodontid species, the guppy *L. reticulatus* where they found that the infection rates of *B. glabrata* snails exposed to approximately 3000 miracidia in presence of the guppy were greatly reduced. Similarly, Upatham and Sturock (1973) in field simulated study, showed that the fish *Poecilia reticulata* used as a decoy, could interfere with and reduce significantly the probability of miracidia infecting *B. glabrata*. Bunnag, (1976) found that in two oxidation ponds harbouring large numbers of *L. reticulatus*, the infection rates of the caged laboratory-bred *B. glabrata* immersed in sewage-polluted water for 5-6 hours, were surprisingly low.

Furthermore, the present study showed that during the experimental period both fish species frequently approached and sometimes fed on the surface of the snails shell and caused them to retract within their shells, reducing the area of exposed body surface. This supports the observation of Bunnag *et al.* (1977) that the guppies were likely to approach and stay around which may feed upon the surface of the snails. Consequently, in natural transmission sites, it may be speculated that, with dispersion of snail intermediate hosts, less miracidial density and large water volume, the fish will greatly interfere with and suppress the infectivity of *S. mansoni* miracidia to the snails . It is evident that fish validation as potential predators may be questioned, especially when miracidial levels are high in heavily contaminated and large volumes of water. On the other hand, there is greater chance of suppression taking place if enough fish and low miracidial levels are presented in the habitat.

Predation of *S. mansoni* miracidia by the fish *O. niloticus* in the laboratory was also studied in the present work. There was active reduction in the number of miracida in all experimental tests (Table 3). In presense of approximately 1500 miracidia, the fish consumed after 60 minutes exposure, from 860 to 1312 with an average of

1066±191.12 miracidia. This represent an average percentage of 71.05 %. In case of approximately 3000 miracida, the number consumed varied between 2040 and 2760 with an average of 2346.75 ± 305.17 (78.2 %). These findings in both tests were in contrast to those observed in the control group which showed no reduction. Thus, the degree of miracidial consumption by fish was directly proportional to miracidia density. These results agree with those of Ismail (1990) who reported that the miracidiophagic activity of *G. affinis* was greatly influenced by miracidial concentration; the higher concentration the more miracidia are consumed by the fish. However, Bunnag *et al.* (1977) stated that there were no appreciable differences in predatory activity of the guppies to the number of miracidia, although the water volume ratio to the miracidial density and the guppies was less when compared to the large water volume in natural habitats. In the present study, the cercariophagic activity of the fish *G. affinis* and *O. niloticus* was clearly demonstrated in laboratory experiments. The number of *S. mansoni* cercariae was dramatically reduced when exposed to fish, and in some experiments no cercariae were remained in the samples of water taken after 60 minutes. With approximately 3000 cercariae, the number consumed by *G. affinis* during one hour ranged from 2600 to 3000 with an average of 2866.7 ± 230.9 (95.6 %), whereas in control test only 6.7% died during the same time. In case of approximately 12000 cercariae, the number consumed by fish ranged from 6400 to 10800 with an average of 9333.3 ± 2540.3cercariae, (77.8 %), whereas in control experiment, the reduction of cercariae was only 13.3 %, (Table 4). On the other hand, the number of cercariae consumed by *O. niloticus* after 60 minutes exposure to about 3000 cercariae varied between 1800 and 2200 with an average of 2066.7±30.9 (68.87 %), in contrast, no reduction of cercariae was found in control test. In presence of about 6000 cercariae, the number consumed by *O. niloticus* ranged from 4000 to 4400 with an average of 4133.3±230.9 (68.9%), whereas in control test, only 6.7 % died during the same time, (Table 5). Thus the predatory action of *G. affinis* and *O. niloticus* on cercariae was increased by increasing number of cercariae. Results of the present work confirm those of other authors (Rowan, 1958; Pellegrino *et al.*, 1966; Knight *et al.*, 1970; Muraleedhoran *et al.*, 1975; Jordan ,1985). The guppy *Lebistes reticulatus* consumed over 7000 *S. mansoni* cercariae in less than 3 hours (Rowan,1958); and when guppies were introduced into spring water containing about 1000 cercariae per fish , a dramatic reduction in the number of cercariae was observed within

60 minutes (Pellegrino *et al*, 1966). Also the fish *Poecilia reticulata* caused a significant reduction in number of cercariae compared to control experiment (Jordan, 1985). Similar observations were obtained by Knight *et al.* (1970) who found that the guppy *L. reticulates* fed on cercariae of *S. mansoni* labeled with radioselenium. Moreover, the present results agree with those of Muraheedharan *et al.* (1975) who reported that the consumption of cercariae by the guppies was related to cercarial concentration.

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Table 1: Percentage infection among groups of 20 *Biomphalaria alexandrina* exposed to *Schistosoma mansoni* miracidia in the presence of *Gambusia affinis.*

Trail No.	Experiment (A) with presence of 10 *G. affinis*			Experiment (B) with presence of 20 *G. affinis*		
	No. of survival snails	No. of infected snails	% infection	No. of survival snails	No. of infected snails	% infection
1	12	1	8.33	14	4	28.57
2	14	4	28.57	15	5	33.3
3	17	3	17.65	15	2	13.3
X ±SD	14.03±2.5	2.7±1.5 ***	18.18	14.7±0.58	3.7±1.5 ***	25.06
Control	14	11	78.57	12	9	75

*** p <0.001 relative control snails.

Table 2: Percentage infection among groups of 20 *Biomphalaria alexandrina* exposed to *Schistosoma mansoni* miracidia in the presence of *Oreochromis niloticus.*

Trail No.	Experiment (A) with presence of 10 *O. niloticus*			Experiment (B) with presence of 15 *O. niloticus*		
	No. of survival snails	No. of infected snails	% infection	No. of survival snails	No. of infected snails	% infection
1	14	6	42.86	13	4	30.77
2	17	7	41.18	19	0.0	0.0
3	18	3	16.67	14	5	35.71
X ±SD	16.3±2.08	5.3±2.08 ***	33.57	15.3±3.21	3±2.65 ***	22.16
Control	18	12	66.67	19	12	63.16

*** p <0.001 relative control snails.

Table 3: Predatory effect of *Oreochromis niloticus* on *Schistosoma mansoni* miracidia.

Experiment (1) with ~ 1500 miracidia			Experiment (2) with ~3000 miracidia		
Miracidia consumed by fish after 60 minutes exposure			Miracidia consumed by fish after 60 minutes exposure		
Trial No.	No.	%	Trial No.	No.	%
1	1100	73.3	1	2227	74.2
2	992	66.1	2	2760	92
3	1312	87.5	3	2040	68
4	860	57.3	4	2360	78.7
X ± SD	1066 ± 191.12	71.05	X ±SD	2346.75 ± 305.17	78.2
Control	0.0	0.0	Control	226 *	7.5

* Number of remaining miracidia in control experiment.

Table 4: Predatory effect of *Gambusia affinis* on *Schistosoma mansoni* cercariae.

Experiment (3) with ~ 3000 cercariae			Experiment (4) with ~ 12000 cercariae		
cercariae consumed by fish after 60 minutes exposure			cercariae consumed by fish after 60 minutes exposure		
Trial No.	No.	%	Trial No.	No.	%
1	3000	100	1	6400	53.3
2	3000	100	2	10800	90
3	2600	86.7	3	10800	90
X ±SD	2866.7 ± 230.9	95.6	X ±SD	9333.3 ± 2540.3	77.8
Control	200 *	6.7	Control	1600 *	13.3

* Number of remaining cercariae in control experiment.

Table 5: Predatory effect of *Oreochromis niloticus* on *Schistosoma mansoni* cerceraiae.

Experiment (5) with ~ 3000 cercariae			Experiment (6) with ~ 6000 cercariae		
cercariae consumed by fish after 60 minutes exposure			cercariae consumed by fish after 60 minutes exposure		
Trial No.	No.	%	Trial No.	No.	%
1	2200	73.3	1	4000	66.7
2	1800	60	2	4400	73.3
3	2200	73.3	3	4000	66.7
X ±SD	2066.7 ± 230.9	68.87	X ± SD	4133.3 ± 230.9	68.9
Control	0.0	0.0	Control	400 *	6.7

* Number of remaining cercariae in control experiment.