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A Small-Scale Field Testing of *Echinostoma liei* as a Biocontrol Agent against *Schistosoma mansoni* in Egypt

FINAL REPORT

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The principal investigator acknowledge with considerable appreciation the technical help and cooperation of all members of the staff of Medical Malacology Department, Theodor Bilharz Research Institute, Cairo.

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A Small-Scale Field Testing of Echinostoma liei as a Biocontrol Agent against Schistosoma mansoni in Egypt.

EXECUTIVE SUMMARY

The objective of this project is to test the potential biocontrol agent Echinostoma liei against Schistosoma mansoni under simulated and natural field conditions in Egypt by obtaining data on : a) The effect E. liei may have on the population density of Biomphalaria alexandrina, the snail host of S. mansoni in Egypt. b) The effect E. liei may exhibit on the infection rate of B. alexandrina with S. mansoni and on the cercariae production by this snail.

Five experiments, of 22 replicates, were carried out between 1988-1990 in similarly controlled ditches at "Snail Research Station" near Cairo. In each experiment/replicate, 5 treatment groups of Biomphalaria snails were introduced into ditches as well as into submerged cages: group (C) without infection, group (S) exposed to Schistosoma, group (E) exposed to Echinostoma, group (S+E) exposed to Schistosoma and less than 2 weeks later to Echinostoma, group (S'+E') to Schistosoma and after 2-3 weeks to Echinostoma. Samples of ditch snails and all caged snails were examined twice weekly until all snails died and the infection rate, duration of cercarial shedding (period of infection risk) and periodic cercarial output were determined. For testing E. liei under natural conditions, four irrigation canals variably infested with B. alexandrina and S. mansoni were provided with maturely developed Echinostoma eggs which were released once or repeatedly along a certain length of each canal. The infection and survival rates of Biomphalaria snails and the number of schistosome infected snails were determined. The experiments were followed up for 3-10 months.

Results show that the infection rate of snails with S. mansoni was significantly lower in group (S+E) and was marginally significantly lower in group (S'+E') than in group (S). Including the zero values, duration of cercarial shedding (infection risk) was significantly shorter in groups (S+E) and (S'+E')than in group (S). The population density of snails became significantly lower in groups (S+E) and (S'+E') than in the control group (C), even though the original densities of snails were similar in these groups. Repeated intervention with E. liei eggs in natural canals resulted in a considerable reduction in population density of B. alexandrina snails, 6 - 8 weeks later and in high suppression in the infection rate of B. alexandrina with S. mansoni.

As a conclusion, E. liei proved to be an efficient biocontrol agent against S. mansoni and its vector B. alexandrina under simulated and natural field conditions. The application would be by dispersing Echinostoma eggs into foci where Schistosoma is mainly transmitted.

The results of this work will be published in 5 papers, three of them are already sent to scientific journals.

RESEARCH OBJECTIVES

Schistosomiasis constitutes an increasing threat to many less-developed countries allover the world and in the combat against this disease, snail control forms an essentiel part. In this respect, the public health authorities in these countries have relied almost exclusively on chemical control strategies. However molluscicides have various disadvantages such as biocid effect to man, animals and plant life, high program expenditures especially with the increasing production costs of molluscicides and possible evolution of resistant strains of snails, etc. This necessitates the search for other control methods as the biological methods.

Echinostoma liei Jeyarasasingam et al (1972) (Trematoda : Echinostomatidae) uses Biomphalaria alexandrina, the vector of Schistosoma mansoni in Egypt, as first intermediate host. With a view to biological control of human schistosomes, Heyneman et al (1972) proved under laboratory conditions that E. liei is a strong dominant competitor over S. mansoni in Biomphalaria glabrata. Echinostoma revolutum (possibly the same as E. liei) has similarly a marked antagonistic effect against S. mansoni in the shared natural snail host B. alexandrina (Baruš et al, 1974). The intramolluscan larval stages of echinostomes usually compete with those of schistosomes leading to death or retarted growth of the later and hence failure to produce cercariae which are infective for man (Basch & Lie, 1966 a & b; Lie, 66, 67, 69; Heyneman &

Umathevy, 1968; Basch et al, 1970). Moreover, all members of the family Echinostomatidae have redia stage which is effective as sterilizing agent and has pathogenic action leading to death of snails. An accessory effect on snails may be also provoked by the metacercariae of echinostomes (Combes, 1982). Meanwhile, E. liei lacks pathogenicity towards man and domestic animals and no human infection has been reported from Egypt.

The objective of this project is to test the potential biocontrol agent Echinostoma liei in the control of the medically important parasite Schistosoma mansoni under simulated and natural field conditions in Egypt by obtaining data on :

a) The effect E. liei may have on the population density of Biomphalaria alexandrina, the snail host of S. mansoni in Egypt.

b) The effect E. liei may exhibit on the infection rate of B. alexandrina with S. mansoni and on the cercariae production by this snail.

This is the first time for testing E. liei against S. mansoni and its snail host B. alexandrina under simulated and natural field conditions in Egypt. In other countries, only six small trials utilizing other echinostomes, schistosomes and snail hosts were performed in Malaysia, Thailand and Guadeloupe (Heyneman &

Umathevy, 1967; Lie et al, 1970, 71, 74 a & b; Nassi et al 1979). All these trials were performed in ponds with various degrees of success and were not costed.

E. liei, if proved successful as a biocontrol agent against S. mansoni and its natural intermediate host in Egypt B. alexandrina, would yield a method of schistosomiasis control with some advantages over present methods i.e. may be safer, more specific and cheaper.

MATERIAL AND METHODS

The test of Echinostoma liei against Schistosoma mansoni under simulated and natural field conditions necessitates the establishing of the life cycle of Echinostoma in the laboratory and the mass production of Echinostoma eggs. This parasite was maintained in B. alexandrina and Physa acuta as first and second intermediate host and the white rat Rattus norvegicus as definitive host. Millions of eggs were usually used at the same time in each experiment.

For testing Echinostoma liei Jeyarasasingam et al in the biological control of Schistosoma mansoni (Sambon) under simulated natural conditions, five experiments, of 22 replicates, were carried out between 1988-1990 in similarly controlled ditches at the experimentation area "Snail Research Station", belonging to Theodor Bilharz Research Institute near Cairo. For the description of this area please see the first publication attached.

In each experiment/replicate, 5 treatment groups of Biomphalaria alexandrina (Ehrenberg) snails were introduced into ditches as well as into submerged cages: group (C) without infection, group (S) exposed to Schistosoma, group (E) exposed to Echinostoma, group (S+E) exposed to Schistosoma and less than 2 weeks later to Echinostoma, group (S'+E') to Schistosoma and after 2-3 weeks to Echinostoma. Samples of ditch snails and all caged

snails were examined twice weekly until all snails died and the infection rate, duration of cercarial shedding (i.e., period of infection risk) and periodic cercarial output were determined.

For testing E. liei as a biocontrol agent under field conditions, six irrigation canals namely El Khassa and Radwan canals (Giza Governorate), Basatin Anshas canal (Sharkiya Governorate), El-Wakf, Sendebis and Sanafeer canals (Qalyoubiya Governorate) were chosen. Three of these canals are typical for secondary canals of the irrigation scheme in schistosomiasis area in Egypt, while the others are typical for tertiary canals. These canals were selected depending on being infested variably with B. alexandrina snails, and S. mansoni and not been molluscicided since years. El Khassa canal contains a relatively low population density of Biomphalaria and a moderate density of aquatic plants. Radwan canal is characterized by a much higher population density of Biomphalaria and dense aquatic vegetation. Basatin Anshas, Sendebis and Sanafeer canals contain a moderate density of Biomphalaria.

Before carrying out the intervention experiments in natural canals, the base line data of snail population dynamics, schistosome and echinostome infections and characteristics of their habitats were obtained. Thus, snails were sampled regularly at monthly intervals for one year during 1988 - 1990. Two snail sampling tools, namely the drag scoop (WHO, 1965) and dip-net (Demian and Kamel, 1972) were used concurrently. The scoop is 100

cm long, 33 cm wide and 25 cm deep, to collect snails in a strip of water from the mid-line of the water course to water edge at the canal bank. The dip net is 33 cm long, 33 cm wide and 8 cm deep to collect snails from the bank to the mid-line of the canal. Snail sampling was performed by taking two passes with each tool adjacent to each other in any sampling site. The sampling sites were taken each 25 m. Using two sampling tools concurrently should provide better representing data of snail population densities and further more allows for comparison of the efficacy of these tools in snail sampling from secondary and tertiary irrigation canals. Snails collected were brought to the laboratory in plastic bags where they were sorted, counted, measured to the nearest millimeter, examined for trematode infection and replaced the next day to their original sites of collection in canals. Examination of snails for natural schistosome and echinostome infections was carried out by exposing snails individually to indirect light in glass tubes containing 2 ml of clean water for 2 and 4 hours respectively and checking them for cercarial shedding. Moreover, a random sample of 10% of collected snails from each canal was crushed and examined for intramolluscan larval stages.

Several physicochemical parameters such as water depth, temperature, pH and conductivity were measured monthly in each canal. Benthos and aquatic vegetation associated with *B. alexandrina* were identified and recorded.

The population density of B. alexandrina was highest in Radwan Canal, therefore its data was utilized for determining the population growth curves and the other parameters of population dynamics. Size frequency histograms were drawn for successive monthly samples of snail populations. The mortality rate and reproductive index of B. alexandrina were computed with the help of the size frequency histograms and the growth curves.

For testing of Echinostoma as a biocontrol agent against S. mansoni and B. alexandrina in natural field conditions, four canals namely Radwan, Basatin Anshas, El Wakf and Sanafeer were used. Thus, in each canal, a length of 100 meter was taken for intervention with E. liei. Thus, maturely developed Echinostoma eggs were released, once or repeatedly, along the median half of the experimental length (50 meters). Another portion 100 meters was taken upstream as control. Snail sampling was carried out using the dip-net each 10 meters. This experiment was followed up for 3 and 10 months in 1990 in Sanafeer and Radwan Canals, for 4 and 7 months in 1991 in El WaKf and Basatin-Anshas canals respectively. The number of positive snails and the infection and the survival rates of Biomphalaria snails were determined.

In the preparatory phase of this project, three snail sampling tools, the dip-net, the drag scoop and a modified Ekman grab, were compared under standarized conditions to estimate numbers of Biomphalaria alexandrina snails of different sizes in narrow

irrigation ditches. Quality control of snail population data depending on double sampling using the same sampling tools was performed in Sendebis and Sanafeer canals. This was carried out the next day of the original sampling by taking 50% of the sampling sites.

RESULTS

Results obtained from experiments under simulated field conditions show that the infection rate of snails with S. mansoni was significantly lower in group (S+E) and was marginally significantly lower in group (S'+E') than in group (S). Including the zero values, duration of cercarial shedding (i.e. infection risk) was significantly shorter in groups (S+E) and (S'+E')than in group (S). However, no significant difference was found in cercarial outout of S. mansoni per shedding snail between various groups. The population density of snails became significantly lower in groups (S+E) and (S'+E') than in the control group (C), even though the original densities of snails were similar in these groups. These results give clear indication that Echinostoma liei reduces the infection of Biomphalaria alexandrina with Schistosoma mansoni and the duration of infection risk.

Intervention with Echinostoma liei eggs in Radwan (Giza), Sanafeer and El Wakf (Qualubiya) and Basatin-Anshas (Sharkiya) canals resulted in a considerable reduction in population density of B. alexandrina snails in these canals, especially when intervention was performed repeatedly. The reduction in snail population was observed after 1.5-2 months. Moreover, snail populations in treated portions of canals did not retain their natural growth and remained less than control till after 3.5 months of intervension. Another effect of the intervention is the highly

suppression which occured in the infection rate of B. alexandrina with S. mansoni. Thus, in the treated portion of Basatin-Anshas canal, the infection rate was deminished to zero in November - December, 1991, meanwhile this rate reached its peak being 25.8% in the control portion.

Other results obtained from the field work show that the patterns of snail spreading along canals are changeable because of water current and irrigation activities. The snail population density in canals showed two seasonal peaks per year in April - May and November - December. Meanwhile, bottoms of snail population were found following after the "Winter Closure" and during the hot summer season. No difference in efficacy was recognized between the dip net and drag scoop when used for snail sampling of Biomphalaria snails in natural secondary and tertiary irrigation canals in Egypt. It was also realized that a quality control system depending on double sampling of 50% of sampling sites the next day is satisfactory in snail population studies.

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PUBLICATIONS OF THE PROJECT

The methods used and results obtained in this project will be published in 5 papers. Three of these publications were already sent to scientific journals and a copy of them is attached. The other two are under final revision and will be sent as soon as possible.

These publications are :

1. Testing of Echinostoma liei as a biocontrol agent against Schistosoma mansoni under simulated natural conditions in Egypt.

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(Published in the Proceedings of Manila Meeting on Aquaculture and Schistosomiasis (6-10 August 1991, arranged by the Board on Science and Technology for International Development (BOSTID)

2. Evaluation of three comon tools in estimating Biomphalaria alexandrina populations in irrigation ditches.

F. Yousif*, M. Khalil**, M. El-Emam*

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(Accepted for publication in the Egypt. J. of Bilharziasis, Cairo)

3. Ecology of Biomphalaria alexandrina the snail vector of Schistosoma mansoni in Egypt.

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(Accepted for publication in J. Egypt. Soc. Parasitol. Cairo)

4. Population dynamics and schistosomal infection of Biomphalaria alexandrina the snail vector of Schistosoma mansoni in Egypt.

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*Medical Malacology Department, Theodor Bilharz Research Institute, P.O.Box 30, Embaba, Cairo. **Department of Zoology, Faculty of Girls, Ain Shams University, Cairo, Egypt. ***Department of Zoology, Faculty of Science, Ain Shams University, Cairo.

(The manuscript under final revision)

5. Intervension with Echinostoma liei against Schistosoma mansoni under natural conditions in Egypt.

F. Yousif, M. El Emam

Department of Medical Malacolgy, Theodor Bilharz Research Institute, P.O. Box 30, Embaba, Cairo.

(Under preparation)

IMPACT OF THE PROJECT FINDINGS ON INTERNATIONAL DEVELOPMENT

The high cost and toxic effects of molluscicides emphasize strongly the need for other approaches of schistosomiasis snail control such as biological methods which may be safer, more specific, cheaper, with relatively permanent results, and have much less harmful effects on the environment. This project on biological control of medically important snails and parasites is a step in this direction. The use of effective biocontrol agents would be however in specific circumstances or as part of integrated control program involving other methods. Cost aspects of this method of biological control is considered in the present project. In addition the methodology and information obtained from this project can benefit several other countries where the disease is endemic, utilizing local echinostomes against local species of schistosomes.

The training aspect is also of significance since several workers from TBRI and the Ministry of Health and Universities in Egypt were associated with the project. They gained considerable traininng and experience in freshwater ecology, snail population studies, parasitology, biological control of snails utilizing echinostomes, etc.

PROJECT PRODUCTIVITY AND FUTURE WORK

The objectives of this project are to test Echinostoma liei against Schistosoma mansoni and its snail vector Biomphalaria alexandrina under simulated and natural field conditions. These tests were carried out by conducting 5 experiments formed of 22 replicates during 3 years under simulated natural conditions and 4 trials during 2 years in 4 natural canals. Data obtained from this work on effect of Echinostoma on population density of Biomphalaria and infection rate of Biomphalaria with Schistosoma and Schistosoma cercarial production give answers for all questions rised in the proposal. Therefore, the goals of this project are amost completely accomplished.

However, before utilizing E. liei in nature further studies are still needed to collect data for the optimization of Echinostoma effects. Thus, the optimum Echinostoma egg dose to be used, best time of the year for application and main effective environmental parameters especially biotic ones are to be determined before efficient application of Echinostoma in nature. Such studies should be carried out under simulated field conditions (in the experimental ditches) as well as on a small scale in the field.

PUBLICATIONS

'20'

Testing of *Echinostoma liei* as a biocontrol agent against *Schistosoma mansoni* under simulated natural conditions in Egypt.

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April 27, 1992

Dr. Fouad Yousif
Medical Malacology Department
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Dear Dr. Yousif:

Greetings! Attached is a copy of your manuscript for the Manila proceedings. Please look it over very carefully as I made considerable editorial changes in certain parts. Make any corrections that you feel are necessary. I tried to be very careful. I hope I did not change any meanings. Be aware that the pagination will change once the proceedings is assembled. For space purposes we dropped one figure.

There are a few specific changes I would like to mention: I reorganized your summary paragraphs slightly to make them stronger. In your References Cited section on page 19 in the first two Lie papers, the second author is listed as both Kwe and Kwo. Which is the correct spelling of the two? Lastly, on page 9, I changed the word "recoded" to "recorded". Is this how you wanted it to read?

I hope you will be pleased with the manuscript. What you see is basically what will be published, with the original figures, by photo offset. Your prompt response will be appreciated.

Congratulations on a good paper!

Sincerely,

Bruce

Bruce A. Harrison
Senior Program Officer

Enclosures

2v

The Board on Science and Technology for International Development (BOSTID) conducts programs with and for developing countries on behalf of the National Research Council, through its Office of International Affairs, which serves the international interests of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

TESTING OF ECHINOSTOMA LIEI AS A BIOCONTROL AGENT AGAINST *SCHISTOSOMA MANSONI* UNDER SIMULATED NATURAL CONDITIONS IN EGYPT[1].

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ABSTRACT

Echinostoma liei was evaluated as a biological control agent of *Schistosoma mansoni* under simulated natural conditions by obtaining data on the effect *E. liei* may have on the population density of *Biomphalaria alexandrina*, the snail host of *S. mansoni* in Egypt and the effect *E. liei* may exhibit on the infection of the snail with *S. mansoni*. Five experiments, of 22 replicates, were carried out between 1988-1990 in similarly controlled ditches at the "Snail Research Station" near Cairo. In each experiment/replicate, 5 treatment groups of snails were introduced into ditches as well as into submerged cages: group (C) without infection, group (S) exposed to *Schistosoma*, group (E) exposed to *Echinostoma*, group (S+E) exposed to *Schistosoma* and (less than 2 weeks later to *Echinostoma*), group (S'+E') to *Schistosoma* and after 2-3 weeks to *Echinostoma*. Samples of ditch snails and all caged snails were examined twice weekly until all snails died and the infection rate, duration of cercarial shedding (i.e., period of infection risk) and periodic cercarial output were determined.

Results obtained show that the infection rate of snails with *S. mansoni* was significantly lower in group (S + E) and was marginally significantly lower in group (S'+E'), than in group (S). Including the zero values, duration of cercarial shedding (i.e., infection risk) was significantly shorter in groups (S + E) and (S'+ E') than in group (S). However, no significant difference was found in cercarial output of *S. mansoni* per shedding snail between various groups. The population density of snails became significantly lower in groups (S+E) and (S'+E') than in the control group (C), even though the original densities of snails were similar in these groups.

INTRODUCTION

Echinostoma liei Jeyarasasingam et al. (1972) uses *Biomphalaria alexandrina*, the vector of *Schistosoma mansoni* in Egypt, as first natural intermediate host. There is considerable evidence demonstrating the value of *E. liei* as a biocontrol agent against *S. mansoni* under laboratory conditions. Thus, Heyneman et al. (1972) proved that *E. liei* is a strong dominant competitor over *S. mansoni* in *Biomphalaria glabrata*. *Echinostoma revolutum* (possibly the same parasite as *E. liei*) proved to have

[1]This research was supported (in part) under Grant No. 936-5542, Program in Science and Technology Cooperation, Office of Research and Development, U.S. Agency for International Development.

marked antagonistic effect against *S. mansoni* in the shared natural snail host *B. alexandrina* (Baruš et al. 1974). Nevertheless, no field tests have been carried out in Egypt to evaluate *E. liei* as a biocontrol agent against *S. mansoni* and its snail vector.

In other countries, field evaluation of echinostomes has been restricted to a few trials in ponds performed in Malaysia, Thailand and Guadeloupe (Lie and Owyang 1973; Heyneman and Umathevy 1967; Lie et al. 1970, 1971, 1974 a,b; Nassi 1978; Nassi et al. 1979; Pointier 1989). In some of these tests, *Echinostoma malayanum*, *Schistosoma spindale* and the snail host *Indoplanorbis exustus* were utilized. In Guadeloupe, *Ribeiroia marini guadeloupensis*, *S. mansoni* and *B. glabrata* were used.

In the present study, *E. liei* was tested against *S. mansoni* and its vector, *B. alexandrina*, in controlled experimental ditches similar to those common in the irrigation system in Egypt. Data on effects of *Echinostoma*, *Schistosoma*, cercarial output, and duration of infection risk were obtained. These data should be of considerable value in the final evaluation of *E. liei* as a biocontrol agent against *S. mansoni*.

MATERIALS AND METHODS

Description of Experimental Ditches

Ten experimental ditches (Figures 1-3) in the Snail Research Station of the Theodor Bilharz Research Institute, 25 km north of Cairo, were utilized for this study. These ditches are parallel, with muddy bottoms and sloping banks, each 30 m long, 150 cm wide at water level, and 50 cm wide at the bottom. The water depth was about 50 cm. The ditches are each divided into 3 sections by special partitions which allow complete separation of sections and water flow without snail mixing. Two channels at right angles to the ditches served as common feeding and draining channels. At the inner side of either channel there is a base in which a series of leveled tubes is installed opposite to the ditches. The tubes of the common feeding channel act as water inlets and are located a few centimeters higher than those of the common drainage channel, which act as outlets to allow the flow of water in one direction. All outlet tubes were guarded by plastic sieves to prevent loss of snails.

This system of experimental ditches is supplied with underground water (40 m deep). The ditches have about 60% of water surface shaded equally. Ditches were originally supplied for two years with surface water from a nearby canal and maintained for natural balance before changing to the underground water source. Vegetation was allowed to grow in all ditches, but partial clearing was necessary to maintain almost equal vegetation density.

Ecological Data

The major abiotic and biotic components were examined. The chemical analysis of the underground and ditch water are presented in Table 1. This analysis shows that both types of water were suitable for the snails and the free-living stages of both parasites. The temperature of surface water at midday and weekly means of temperatures at the middle and the bottom of ditches were determined using a thermograph (Figure 4). The mean water temperature was 16°C - 26°C during the period of experiments (March - November). The period from December to February was avoided since water temperatures are low, the development null points for *S. mansoni* and *E. liei* are 14°C and

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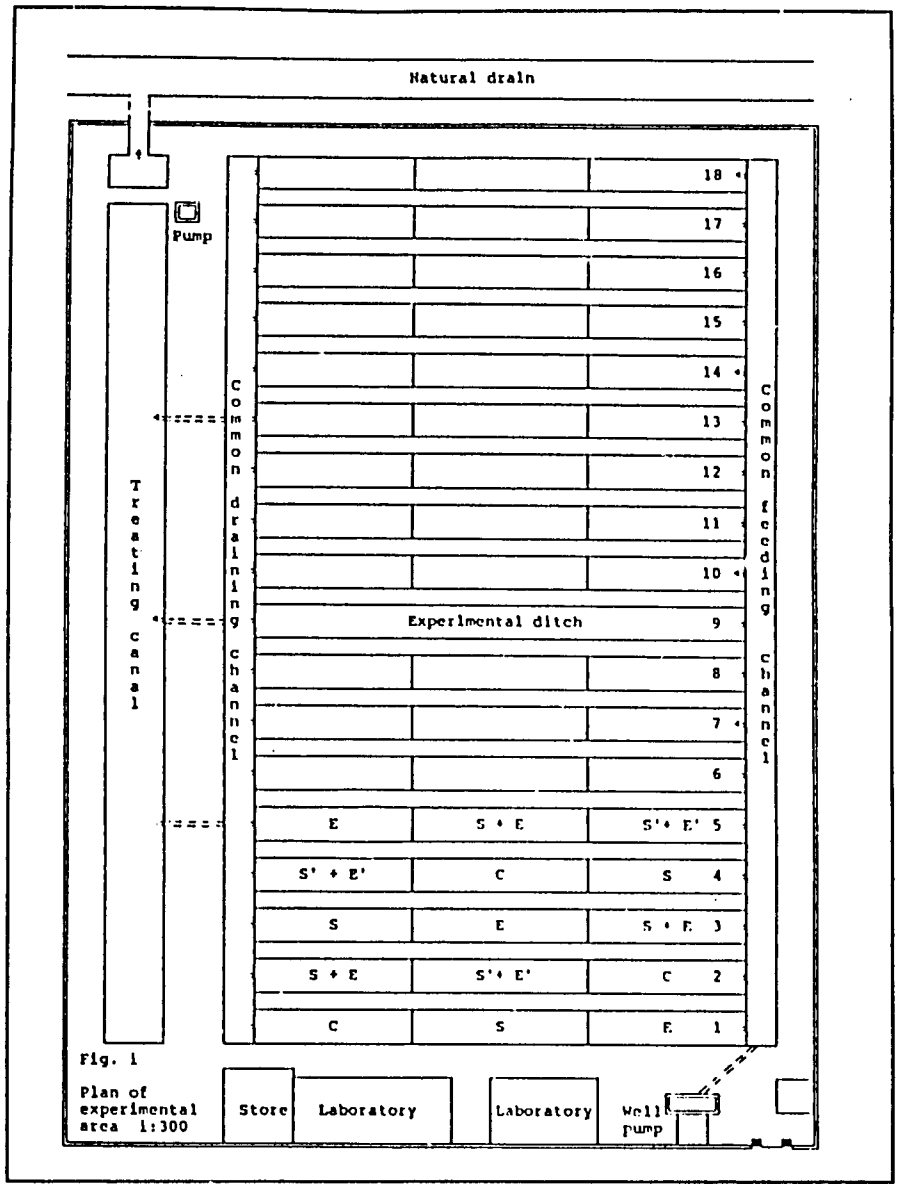

Fig. 1
Plan of experimental area 1:300

FIGURE 1. Plan of experimental area 1:300.

13°C, respectively (Pflüger 1980, Yousif et al., unpublished). In the cool season, December to February, the mean water temperature at the bottom of the ditches (14°C -16°C) is higher than at the middle by about 2°C. The reverse occurs during the rest of the year, the mean temperature at the bottom of the ditches is 1°C - 3°C lower than that at halfway to the bottom. Surface water temperature at midday was about 3°C lower than the mean temperature at the bottom during January. This temperature was higher by up to 5°C than the bottom temperature in December and February.

In the warm season, March - November, the temperature of water surface at midday was higher than the mean at the bottom by 7°C - 12°C in June - August. The fauna and flora in various ditches (see below) were generally similar both in species and density.

Fauna and Flora in Ditches

Insects
Lethocerus sp.
Dolomedes sp.
Anisops sp.
Anisoptera nymph

Amphibians
Tadpoles of *Bufo* sp.

Fish
Tilapia nilotica

Aquatic Plants
Eichhornia crassipes
Cyperus dives
Phragmites australis
Phragmites communis
Typha domingensis
Paspalum paspaloides
Ceratophyllum demersum
Chara sp.

Mollusks
Bulinus truncatus
Physa acuta
Lanistes carinatus
Cleopatra bulimoides
Lymnaea caillaudi
Bellamya unicolor

Filamentous algae

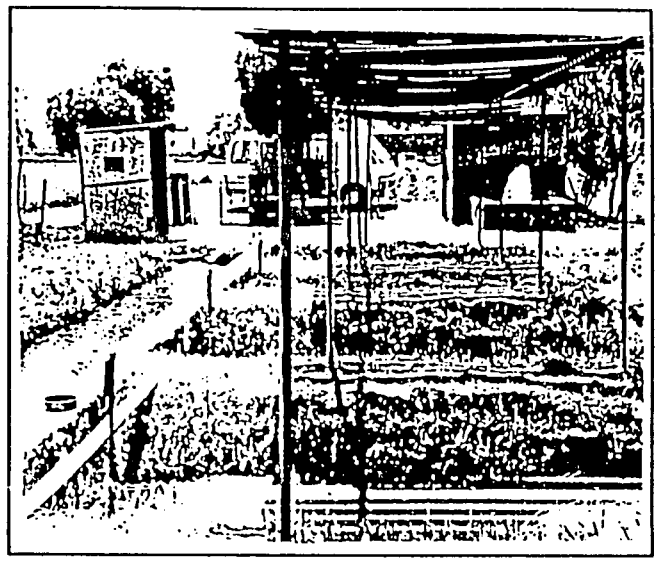

FIGURE 2. The experimental ditches in "Snail Research Station" near Cairo.

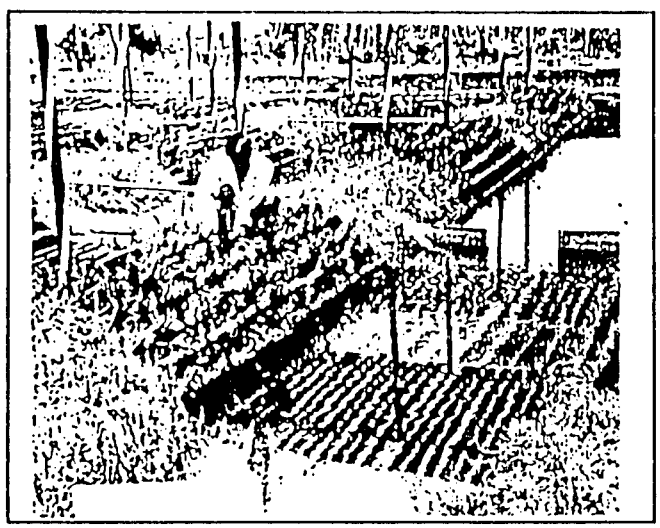

FIGURE 3. An experimental ditch and opened dividing partition.

TABLE 1. Chemical analysis of underground and ditch water.

Parameter (ppm)	Underground water	Ditch water
Sulfides (S)	0.1	0.36 (0.16 - 0.58)
Dissolved oxygen (O_2)	0.7	
Biochemical oxygen demand (B.O.D.)	4.2	3.68 (2.00 - 9.40)
Chemical oxygen demand (C.O.D.)	8.0	< 15
Total dissolved solids (T.D.S.)	600	496.7 (486 - 503)
Ash of dissolved solids	562	
Suspended solids	66	
Ash of suspended solids	40	
Ammonia (N)	1.37	0.01 (0.01 - 0.02)
Nitrites (N)	0.03	0.30 (0.07 - 0.23)
Nitrates (N)	0.51	0.26 (0.01 - 2.10)
Oils and fats	Nil	
Iron (Fe)	0.6	0.57 (0.50 - 0.65)
Manganese (Mn)	1.5	0.99 (0.90 - 0.75)
Total alkalinity ($CaCO_3$)	290	230.50 (214 - 248)
Total hardness ($CaCO_3$)	284	275 (226 - 302)
Calcium hardness ($CaCO_3$)	188	153.50 (110 - 176)
Magnesium hardness	116	121.50 (108 - 136)
Calcium (Ca)	76.2	65.51 (60.44 - 70.40)
Magnesium (Mg)	27.8	29.13 (27.8 - 32.60)
Chlorides (Cl)	132	136 (136 - 136)
Silicates (SiO_2)	28.1	0.40 (0.40 - 0.40)
Phosphates (PO_4)	0.7	
Copper (Cu)	Nil	
Lead (Pb)	0.05	
Faecal coliforms/100 c.c.	24	62.8 (12 - 128)

Specimens required for this research were *B. alexandrina* snails, *E. liei* eggs and *S. mansoni* eggs. Snails were collected from canals and drains near Cairo, examined successively for natural infection, and shedding snails were discarded. In each experiment, batches of collected snails were random'y distributed in equal numbers between different sections of the ditches. Only medium-sized snails (5-8 mm in diameter) were used.

Echinostoma liei and *S. mansoni* were maintained in the laboratory for the production of eggs. Both parasites originated from naturally infected *B. alexandrina* snails collected from the irrigation scheme near Cairo. *Echinostoma liei* was maintained in albino rats (*Rattus norvegicus*) as definitive host, *B. alexandrina* and *Physa acuta* as first and second intermediate hosts, respectively. Egg production of both parasites was optimized and a technique was refined for egg extraction by passing a faecal suspension or tissue homogenate through a number of sieves of descending mesh sizes. Eggs were incubated and only fully embryonated eggs ready to hatch were used. *Schistosoma mansoni* was maintained in golden hamsters (*Mesocricetus auratus*) and *B. alexandrina* snails. An extra source for *Schistosoma* eggs from human origin was also utilized.

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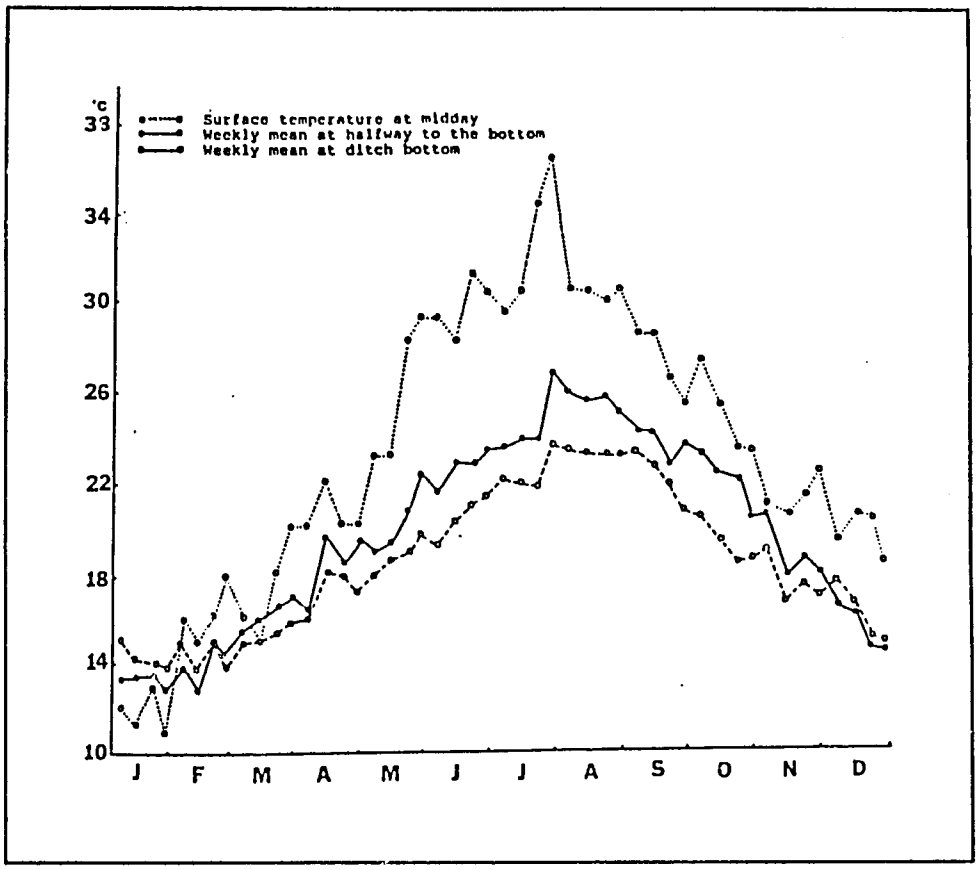

FIGURE 4. Water temperature of experimental ditches during 1990.

Five experiments of 22 replicates were set up in years 1988-1990 (Table 2). In each experiment/replicate, five treatments were used. These groups are: group (C) snails without infection, group (S) snails exposed to *Schistosoma* eggs, group (E) snails exposed to *Echinostoma* eggs, group (S + E) snails exposed to *Schistosoma* and to *Echinostoma* after less than 2 weeks, group (S'+ E') snails exposed to *Schistosoma* and to *Echinostoma* after 2-3 weeks. Thus, in each experiment/replicate, (except those of experiment 3) 5 equal groups of snails were introduced into five similarly controlled ditch sections. Simultaneously, 150 or 300 snails were placed in 3 cages and submerged in each ditch section. A rotation technique for the distribution of groups in various sections was applied (Figure 1). Therefore, the effect of the position of the section within the ditch was eliminated. In the replicates of experiment 3, equal numbers of snails were introduced into the ditch sections during the previous autumn and maintained until the next spring. Before introducing *Schistosoma* and *Echinostoma* eggs, the snail population in each ditch section was assessed by counting the number of snails obtained by 12 dips, using the standard dip net.

This assessment showed that the snail populations in ditch sections of replicates 4 and 6 were considerably lower than in replicates 1,2,3, and 5. Therefore, 2000 and 500 snail/section were added to the former replicates respectively.

TABLE 2. Intervention by *Echinostoma liei* against *Schistosoma mansoni* under simulated field conditions: setting up of experiments.

Exp. no.		Date	Snails (1000)	Number/ditch section Schistosoma Eggs (1000)	Echinostoma Eggs (1000)
I	1	May, 88	4	100	1,000 - 1,425
	2	May, 88	4	46	500 - 940
	3	May, 88	4	43	800
II	1	Aug. 88	3	54	545
	2	Sep. 88	3	35	780 - 975
	3	Sep. 88	3	71	700 - 950
	4	Oct. 88	3	74	697
III	1	Mar. 89		72	1,000
	2	Mar. 89		87	1,000
	3	Apr. 89		84	1,000
	4	Apr. 89		69	1,500
	5	May, 89		116	1,000
	6	Jun. 89		102	1,000
IV	1	Aug. 89	5,2	100	1,000
	2	Aug. 89	5,2	97	1,000
	3	Sep. 89	5,2	108	1,000
V	1	May, 90	5,4	104	1,000
	2	May, 90	5,4	96	1,000
	3	May, 90	5,4	91	1,000
	4	May, 90	5,4	120	1,000
	5	Jun. 90	5,4	100	1,000
	6	Jul. 90	5,6	98	1,000

Eggs were introduced into ditches at midday, using shallow pots hung 5 cm under the water surface and left for 5 days in the case of *Echinostoma*, and one day in the case of *Schistosoma*. Ditch sections were separated from each other using impermeable screens during periods of exposure to miracidia to prevent miracidial contamination between sections. Samples of ditch snails (12 dips/ditch section and all caged snails) were examined twice weekly until the death of all the introduced snails. These snails were examined individually for cercarial shedding every two hours. Positive snails were marked according to parasite species, and all snails were replaced into their original sites. The infection rate, duration of cercarial shedding in each group (i.e., period of infection risk), and periodic cercarial output per shedding snail were determined in each case.

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Statistical Analysis

The primary analyses methods used for this project were multiple logistic regression and multiple regression. Additionally, the non-parametric median test was used for certain comparisons of highly skewed data. All analyses were conducted using the statistical software package "Statistix" for personal computers. In all analyses directed toward determining factors related to infection of *Schistosoma* or *Echinostoma* in snails, multiple logistic regression was used. When making comparisons across treatment groups (or allowing for differences across experiments, experiment/replicates and/or treatment groups), the appropriate dummy variables were created.

TABLE 3. Infection of *Biomphalaria alexandrina* with *Echinostoma liei* under simulated field conditions (a: in ditches).

Exp. no.	Snails exposed to: Echinostoma (E) alone			Schistosoma + (E) (< 2 weeks)			Schistosoma + (E) (2-3 weeks)		
	Examined	+ve	%	Examined	+ve	%	Examined	+ve	%
I 1				91	15	16.4	112	7	6.2
3	52	3	5.7	74	7	9.4	1	0	0
Total	52	3	5.7	165	22	13.3	113	7	6.2
II 1	18	1	5.5	49	14	28.5	31	2	6.4
2	92	11	11.9	62	8	12.9	83	12	14.4
3	34	4	11.7	9	0	0	6	1	16.6
4	18	2	11.1	139	0	0	2	0	0
Total	162	18	11.1	259	22	8.4	122	15	12.3
III 1	399	28	7.0	338	34	10.0	276	22	7.9
2	213	22	10.3	221	19	8.5	280	10	3.5
3	152	5	3.2	89	2	2.2	138	8	5.7
4	88	17	19.3	64	13	20.3			
5	112	6	5.3	30	6	20.0			
6	32	2	6.2	20	0	0	19	0	0
Total	996	80	8.5	762	74	9.7	713	40	5.6
IV 1	113	2	1.7	97	2	2.0	112	6	5.3
2	34	7	20.5	85	7	8.2	158	0	0
Total	147	9	6.1	182	9	4.9	270	6	2.2
V 1	76	1	1.3	76	1	1.3	61	2	3.2
2	47	5	10.6	91	8	8.7	188	17	9.0
3	133	6	4.5	125	7	5.6	100	2	2.0
4	197	14	7.1	144	12	8.3	130	14	10.7
5	80	10	12.5	102	14	13.7	83	0	0
6	88	3	3.4	79	1	1.2	85	0	0
Total	621	39	6.3	617	43	7.0	647	35	5.4
G.total	1978	149	7.5	1985	170	8.6	1865	103	5.5

In determining whether there was a correlation between the infection rates of snails in ditches with those in cages in the same treatment group, multiple regression was used; in some cases, the data were skewed, so that a logarithm transformation was first made. (In such cases, zero values were recoded to one-half the lowest positive value.) When the non-parametric median test was employed, it is indicated in the appropriate place of the paper.

RESULTS

Echinostoma infection

a. Snails in ditches (Table 3)

After allowing for differences in the shedding rates of *Echinostoma* in snails, among the five experiments, this rate in group (S'+ E') was significantly lower than in group (S + E) (one-tailed $p = 0.006$). The probability of infection in group (S + E) was not significantly different from that in group (E). However, the probability for group (S'+ E') was significantly lower than for group (E) (one-tailed $p = 0.027$).

When data from all three snail groups exposed to *Echinostoma* were combined, the probability of infection was significantly and positively related to the number of *Echinostoma* eggs introduced (one-tailed $p = 0.006$); the number of eggs remained significant (one-tailed $p = 0.01$) after allowing for differences in infection across the three groups. However, when the three groups were analyzed separately, the number of eggs was a significant factor only for group (E) (one-tailed $p = 0.006$).

b) Snails in cages (Table 4)

As with the data from ditch snails, the prevalence of infection (cercarial shedding) in caged snails was significantly lower in group (S'+ E') than in group (S + E) (one-tailed $p < 0.0002$). The probability of infection for group (S + E) was not significantly different from that in group (E), but was significantly lower in group (S'+ E') compared with group (E) (one-tailed $p = 0.0004$).

For snails in cages, (cf. ditch snails), the numbers of *Echinostoma* eggs introduced were negatively correlated with the probability of infection, after controlling for differences across groups ($p < 0.0001$). When the three treatment groups were analyzed separately, these inverse relationships were significant for groups (S + E) and (S'+ E'), but only marginally for group (E) (two-tailed $p = 0.13$).

There were 17 experiments/replicates in which snails from both ditches and cages were examined in sufficient numbers for *Echinostoma* infection. The infection rates (as well as their logarithms, to account for non-normality) were positively correlated (one-tailed $p = 0.026$).

TABLE 4. Infection of *Biomphalaria alexandrina* with *Echinostoma liei* under simulated field conditions (b: in cages).

Exp. no.	Snails exposed to: Echinostoma (E) Alone			Schistosoma + (E) (< 2 weeks)			Schistosoma + (E) (2-3 weeks)		
	Examined	+ve	%	Examined	+ve	%	Examined	+ve	%
I 1				47	7	14.8	40	1	2.5
2	43	10	23.2	38	10	26.3	3	0	0
3									
Total	43	10	23.2	85	17	20	43	1	2.3
II 1	23	1	4.3	30	15	50	37	0	0
2							4	4	100
3	64	30	46.8	40	15	37.5	33	14	42.4
4	12	7	58.3	9	0	0	10	3	30
Total	99	38	38.3	79	30	38	84	21	25
III 1	107	2	1.8	149	4	2.6	116	0	0
2	68	8	11.7	43	11	25.5	59	6	10.1
3	72	25	34.7	37	12	32.4	49	4	8.1
4	44	14	31.8	73	8	10.9	–		
5	15	0	0	37	3	8.1			
6	97	3	3.0	77	1	1.4	56	0	0
Total	403	52	12.9	416	39	9.4	280	10	3.6
IV 1	103	4	3.8	111	10	9.0	109	5	4.5
2	60	18	30	64	0	0	90	3	3.3
3	50	32	64	50	9	18	22	5	22.7
Total	213	54	25.4	225	19	7.4	221	13	5.9
V 1	228	11	4.8	178	5	2.8	240	3	1.2
2	124	6	4.8	164	10	6.0	335	15	4.4
3	330	20	6.0	270	23	8.5	300	2	0.6
4	530	22	4.1	280	17	6.0	82	18	21.9
5	260	15	5.7	95	16	16.8	90	9	10
6	290	13	4.4	196	16	8.1	244	16	6.5
Total	1762	77	4.4	1183	87	7.4	1291	63	4.9
G.total	2520	231	9.2	1988	192	9.7	1919	108	5.6

Schistosoma Infection

a. Snails in ditches (Table 5)

In the five experiments, the percentage of infected (shedding) snails in group (S'+ E') was statistically higher than in group (S + E). Therefore, the two groups cannot be combined. In these analyses, data from experiment I, replicate 3 were deleted; it was judged that too few snails were examined in group (S) and (S'+ E').

TABLE 5. Effect of *Echinostoma liei* on infection of *Biomphalaria alexandrina* with *Schistosoma mansoni* under simulated field conditions (a: in ditches)

Exp. no.	Snails exposed to: Schistosoma (S) Alone			(S) + Echinostoma (< 2 weeks)			(S) + Echinostoma (2-3 weeks)		
	Examined	+ve	%	Examined	+ve	%	Examined	+ve	%
I 1	122	14	11.4	91	7	7.6	112	15	13.3
3	4	0	0	74	0	0	1	1	100
Total	126	14	11.1	165	7	4.2	113	16	14.2
II 1	108	5	4.6	49	2	4.0	31	0	0
2	40	0	0	62	0	0	83	2	2.4
3	27	1	3.7	9	1	11.1	6	1	16.6
4	137	5	3.6	139	4	2.8	2	0	0
Total	312	11	3.5	259	7	2.7	122	3	2.6
III 1	351	2	0.6	338	1	0.2	276	0	0
2	297	4	1.3	221	0	0	280	0	0
3	125	0	0	89	0	0	138	0	0
4	76	1	1.3	64	0	0			
5	317	10	3.1	30	0	0	31		
6	20	1	5	20	0	0	19	0	0
Total	1186	18	1.5	762	1	0.1	713	0	0
IV 1	151	1	0.6	97	0	0	112	1	0.8
2	42	4	9.5	85	2	2.3	158	11	6.9
Total	193	5	2.6	182	2	1.1	270	12	4.4
V 1	69	2	2.8	76	0	0	61	0	0
2	149	7	4.6	91	4	4.3	188	9	4.7
3	106	1	0.9	125	1	0.8	100	0	0
4	194	11	5.6	144	4	2.7	130	5	3.8
5	87	8	9.1	102	3	2.9	83	5	6.0
6	113	4	3.5	79	0	0	85	0	0
Total	718	33	4.6	617	12	1.9	647	19	2.9
G.total	2535	81	3.2	1985	29	1.5	1865	50	2.7

Differences across the five experiments as well as across experiment/replicates were allowed. Results were nearly identical regarding differences across groups. Because there were clear differences across replicates within some of the experiments, differences across experiment/replicates was appropriate. After allowing for these replicate differences, the percentage of infected snails was significantly lower (47%) in group (S + E) than in group S (one-tailed $p = 0.0005$) and was marginally significantly lower in group (S'+ E') than in group (S) (one-tailed $p = 0.65$).

When all three groups were combined, the number of eggs introduced was a highly significant factor in the probability of infection in snails ($p < 0.0001$). When the three treatment groups were analyzed separately, the number of eggs was either significant as in groups (S) and (S'+ E'), one-tailed p-values < 0.01) or marginally significant group as in (S + E), (one-tailed $p = 0.056$). In all

cases, the probability of infection increased with increasing number of eggs. Because of the small number of snails counted in either one or both of the treatment groups, the data from experiment I, replicate 2 and experiment II, replicate 2 were not included in these analyses.

b. Snails in cages (Table 6)

The same approach was used as with the snails in the ditches; i.e., differences in infection rates across experiment/replicates were allowed for before assessing the effect of the treatment group.

TABLE 6. Effect of Echinostoma liei on infection of Biomphalaria alexandrina with Schistosoma mansoni under simulated field conditions (b: in cages).

Exp. no.	Snails exposed to: Schistosoma (S) Alone			(S) + Echinostoma (< 2 weeks)			(S) + Echinostoma (2-3 weeks)		
	Examined	+ve	%	Examined	+ve	%	Examined	+ve	%
I 1	50	8	16	47	12	25.5	40	11	27.5
2	14	3	21.4	38	0	0	3	1	33.3
Total	64	11	17.2	85	12	14.1	43	12	27.9
II 1	63	7	11.1	30	1	3.3	37	7	18.9
2	8	0	0				4	0	0
3	6	2	33.3	40	1	2.5	33	4	12.1
4	17	0	0	9	0	0	10	0	0
Total	94	9	9.6	79	2	2.5	84	11	13.0
III 1	109	3	2.7	149	0	0	116	0	0
2	150	1	0.6	43	0	0	59	0	0
3	42	1	2.3	37	0	0	49	0	0
4	25	1	4	73	0	0			
5	96	10	10.4	37	0	0			
6	33	14	42.4	77	0	0	56	1	0
Total	455	30	6.5	416	0	0	280	1	0.3
IV 1	165	0	0	111	2	1.8	109	2	1.8
2	73	14	19.1	64	3	4.6	90	19	21.1
3	75	36	48	50	5	10	22	3	13.6
Total	313	50	15.9	225	10	4.4	221	24	10.9
V 1	177	4	2.2	178	0	0	240	0	0
2	333	8	2.4	164	4	2.4	335	4	1.1
3	113	2	1.7	270	1	0.3	300	0	0
4	245	23	9.3	280	8	2.8	82	8	9.7
5	262	15	5.7	95	6	6.3	90	6	6.6
6	300	13	4.3	196	3	1.5	244	6	2.4
Total	1390	42	4.6	1183	22	1.9	1291	24	1.9
G.total	2316	129	7.1	1988	46	2.3	1919	72	3.8

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The probability of infection for snails in group (S + E) was significantly lower (32%) than those in group S (one-tailed $p < 0.0001$), but was not significantly lower in group (S'+ E') compared with group S. In these analyses of snails in the ditches and in cages, the coefficients for group (S + E) were very similar: -.79 for ditches, and -.80 for cages. Using the value of -.80, the ratio of the odds of infection for snails in group (S + E) relative to group (S) is .45. Thus, for an odds ratio of .45, if the prevalence of infection among group (S) is, for example, 4%, then the prevalence among group (S + E) would be estimated as approximately 1.8%.

When data from all three groups were combined, the probability of infection was significantly and positively related to the number of *Schistosoma* eggs introduced (one-tailed $p = 0.024$); the number of eggs remained significant (one-tailed $p = 0.036$) after allowing for differences in infection across the three groups. However, when the three groups were analyzed separately, the number of eggs was a significant factor only for group (S + E) (one-tailed $p = 0.014$). There were 17 experiment/replicates in which snails from both ditches and cages were examined in sufficient numbers for *Schistosoma* infection. The infection rates (as well as their logarithms, to account for non-normality) were highly correlated ($p < 0.0001$, $r = 0.42$).

Effect of *Echinostoma* on periodic cercarial output/shedding snail: (Table 7)

Because of the very small number of positive snails for some replicates, results within replicates of the same experiment were combined. Data from experiment III were not included in the analyses since only one snail from the treated groups for this experiment were observed to be positive. Multiple regression, both unweighted and weighted by the number of infected snails, was used to compare the numbers of cercariae/snail in the treated groups (S + E) and (S'+ E') with the *Schistosoma* group (S). Because the means of each experiment/group had differing precision because of the different numbers of snails contributing to these means, the weighted regression was more appropriate.

For snails in the ditches, when averaged across the four experiments, the mean number of cercariae/shedding snail/2h was lower in group (S'+ E') than for the *Schistosoma* alone group (S). However, this difference was not statistically significant; moreover, for two of the experiments the mean was higher in group (S'+ E') than group (S). There was also no significant difference between group (S + E) and group (S).

For snails in cages, the mean numbers of cercariae/shedding snail/2h were significantly lower in both groups (S + E) and (S'+ E') than in group (S) (one-tailed p-values 0.015 and 0.026, respectively) for unweighted regression. However, when the number of snails was considered, using weighted regression, the difference did not approach statistical significance. The great majority of infected snails were observed in experiments IV and V. For these two experiments, the means across treatment groups are similar (with exception of experiment IV, treatment group 4, which also was based on the smallest number of snails).

Thus, since the unweighted results are considered more reliable, it is concluded that for both ditches and cages, there were no differences in the periodic cercarial output/shedding snail in various treatment groups.

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TABLE 7. Effect of *Echinostoma liei* on cercarial output of *Schistosoma mansoni* under simulated field conditions.

Exp. no.	Schistosoma (S) Alone		(S) + Echinostoma < 2 weeks		(S) + Echinostoma 2-3 weeks	
	Cercariae/Snail/2h		Cercariae/Snail/2h		Cercariae/Snail/2h	
	Ditches	Cages	Ditches	Cages	Ditches	Cages
I 1	86.5	167.6	124.8	76.2	61	32.6
2		280				219
3					30	
II 1	169.5	110.4	4.5	23		25.6
2					62.5	
3	13	182.5	20	40	52	151.2
4	247.5		199.3			
III 1	106	76.5	85			
2	32.5	64.8				
3		42				
4	80	83.3				
5	72	85.2				
6	50	42.5				100
IV 1	37				62	160
2	88	82.1	65	140	92.5	111.2
3		106.8		43.7		20.8
V 1	75	86.7				
2	97	128.9	85.7	102.1	137	95.7
3	27.5	21.2	10	25		
4	60.2	62.9	77.1	52	103	62.9
5	90	78.7	80	75	88	83.3
6	11	32.8		16.7		53.9
Mean	83	88.1	98.5	67.2	90.3	86

Duration of *Schistosoma* infection risk (Table 8)

The data concerning days of cercarial shedding for various snail groups (days of infection risk) in ditches and cages were highly skewed, with many zero values. Therefore, in comparing each of the treatment groups (S + E) and (S'+ E') with group (S), the non-parametric median test for paired data was used. In this, for the 20 experiment/replicates, the number of times the duration of shedding in group (S) exceeded the other groups (S + E) and (S'+ E') was noted; based on the binomial distribution, the exact p-value was calculated. (Note: under the null hypothesis of no difference in the number of shedding days, for approximately half the replicates, the number of days for group S should be larger than for the other groups and for half the replicates it should be smaller.)

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For both ditches and cages, both groups (S + E) and (S'+ E') were found to have significantly fewer days of cercarial shedding i.e., shorter period infection risk. In the ditches, the duration of *Schistosoma* infection risk is 49% in group (S + E) and 57% in group (S'+ E') of that of group (S). The respective one-tailed p-values were: group (S + E) ditches 0.048; group (S'+ E') ditches 0.0004; group (S + E) cages 0.004; group (S'+ E') cages 0.0008.

TABLE 8. Effect of *Echinostoma liei* on mean period in days of *Schistosoma* infection risk.

Exp. no.		Schistosoma (S) Alone		(S) + Echinostoma < 2 weeks		(S) + Echinostoma 2-3 weeks	
		Ditches	Cages	Ditches	Cages	Ditches	Cages
I	1	18	11	10	8	30	4
	2		8		0		4
	3	0		0		4	
II	1	14	7	4	4	0	6
	2	0	0	0	0	18	0
	3	4	15	8	4	4	26
	4	15	0	18	0	0	0
III	1	33	54	4	0	0	0
	2	22	18	0	0	0	0
	3	0	4	0	0	0	0
	4	4	20	0	0	0	0
	5	11	50	0	0	0	0
	6	4	43	0	0	0	4
IV	1	4	0	0	4	4	23
	2	28	14	11	4	43	76
	3		34		20		20
V	1	4	18	0	0	0	0
	2	11	24	24	20	20	20
	3	4	8	4	4	0	0
	4	22	71	31	22	22	42
	5	23	16	4	4	19	11
	6	18	41	0	27	0	28
Mean		11.9	21.7	5.9	5.7	8.2	12.5

Effect of *Echinostoma* on the population density of snails (Table 9)

Data obtained from experiment III is used here since the snail populations were more natural than in other experiments (see Materials and Methods). In order to assess the original conditions for each treatment group (E, S + E, S'+ E'), the original snail densities estimated by the number of snails recovered in 12 dips, were compared with the control group (C). Because of non-normality of the data, the non-parametric median test was used. None of the three treatment groups differed significantly from the control.

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TABLE 9. Effect of *Echinostoma liei* on the population density of *Biomphalaria alexandrina* under simulated field conditions.

Exp. no.	Observation period (days)	Control		Echinostoma (E) Alone		Schistosoma + (E) (< 2 weeks)		Schistosoma + (E) 2-3 weeks	
		Original density (snails/ 12 dip)	Number of collected snails	Original density (snails/ 12 dip)	Number of collected snails	Original density (snails/ 12 dip)	Number of collected snails	Original density (snails/ 12 dip)	Number of collected snails
III									
1	69	49	388	29	399	128	338	99	276
2	53	42	336	40	213	38	221	37	280
3	43	78	230	104	152	59	89	88	138
4	45	18 (+2000)*	185	20 (+2000)*	88	15 (+2000)*	64	—	—
5	63	62	179	33	112	39	30	48	37
6	77	32 (+ 500)*	96	28 (+ 500)*	32	42 (+ 500)*	20	30 (+ 500)*	19

* Number of snails added to each ditch section at start of experiment.

For comparing the population density of snails, two measures were considered: the number of snails collected during the experiment and the ratio of the number of snails collected to the original density. For groups (S + E) and (S'+ E') the results were identical for the two measures. The control group (C) exceeded group (S + E) for all six experiments and exceeded group (S'+ E') for all five experiments in which data were collected for both. These differences were statistically significant; one-tailed $p = 0.02$ and $p = 0.03$ for groups (S + E) and (S'+ E'), respectively. The numbers collected for group (E) tended to be lower than for group (C); the mean number of snails collected for groups (E) and (C) were, respectively, 166.0 and 235.7, and the number collected in the control group exceeded that for group (E) in five of the six experiments. However, these differences were not significant ($p = 0.11$). When the ratios of collected snails to original densities were considered, the values for group (E) again tended to be lower than group (C) (mean ratios of 4.9 and 5.8, respectively, with the ratio in the control group exceeding that in group (E) for four of the six experiments, but these differences were not significant.

DISCUSSION

Echinostoma liei was tested here for the first time against *Schistosoma mansoni* and the shared snail host *Biomphalaria alexandrina* under simulated natural conditions, and in controlled experimental ditches similar to those common in the irrigation schemes in schistosomiasis areas of Egypt. Previous field trials with echinostomes for control of schistosomes are limited to those performed in Malaysia, Thailand and Guadeloupe using *E. malayanum* against *S. spindale*, and the snail host *Indoplanorbis exustus* and *Ribeiroia marini* against *S. mansoni* and *B. glabrata*, respectively. Some of these trials proved that echinostomes are generally dominant over schistosomes, and will almost eliminate schistosomes from the ponds, and are effective in reducing mollusk populations (Heyneman and Umathevy 1967; Lie et al. 1970, 1971, 1974a,b; Nassi 1978; Lie and Owyang 1973; Nassi et al. 1979; Pointier 1989).

Schistosoma mansoni, *E. liei* and the shared snail vector *B. alexandrina* used in the present study were originally obtained from the same locality. This was meant to avoid any effect of strain difference on susceptibility of the snails to either parasite. Snails used in experiments were always of the same medium size to neutralize the effect of snail size on susceptibility to infection. *Echinostoma* eggs were incubated before being used in the experiments. Fully embryonated readily hatchable eggs were placed in pots and not simply thrown into ditch water. This avoided the settling of eggs on the bottom of the ditches and their being buried in mud or ingested by other organisms.

Conditions in the experimental ditches were kept as uniform as possible, especially water flow, depth and quantity, type and density of vegetation and shade. Meanwhile the number of experiments/replicates were numerous (22), including negative and positive controls, to allow for statistical analyses of the results.

The results show that the infection of snails with *Echinostoma* was significantly lower in group (S'+ E') than in groups (E) and (S + E) in both ditches and cages. Infection of snails with *Echinostoma* in the last two groups was not different. This lower infection rate with *Echinostoma* in group (S'+ E') was expected since *Echinostoma* eggs were introduced into this group about 10 days later than into the other groups. Infections with *Echinostoma* in caged snails was found to be significantly negatively correlated to the number of introduced eggs of the parasite, while positively

correlated in ditch snails. .This may be explained on the basis of the higher exposure of cage snails to echinostome miracidia leading to a higher mortality of infected snails when compared to ditch snails. Such effects have not been observed in the case of *Schistosoma* because of the much less pathogenic effect of this parasite on vector snails in comparison with *Echinostoma*.

The results also show that the infection rate of ditch snails with *Echinostoma* or *Schistosoma* ranged between 5.7% -11.1% (7.5%) and 1.5% - 11.1% (3.2%) in positive control groups, i.e., groups (E) and (S), respectively. These averages are somewhat higher than the averages of natural infection rates for both trematodes in Egypt. Much higher infection rates of snails with these parasites may be obtained under laboratory conditions, but this appears not to be the case under simulated natural conditions.

Intervention with *Echinostoma* should be by introducing completely developed eggs into foci where *Schistosoma* is transmitted. It appears from the results of this study that the timing of this intervention with echinostomes is an important factor to consider. Thus, a period less than two weeks between schistosome infection of snails and echinostome intervention was necessary to demonstrate a significant effect in the present study. The optimization of this factor still needs further studies.

This study has demonstrated that a single application of *Echinostoma liei* eggs can have a significant reducing effect on *B. alexandrina* populations that were previously exposed to *S. mansoni*. Moreover, this application can significantly suppress the schistosome parasite in snails and shorten the period of infection risk, if it occurs less than 2 weeks from the exposure of the snails to the schistosome. These effects are additive and consequently, *E. liei* can provide a biological method of schistosomiasis control with some advantages over the present chemical methods, especially if applied repeatedly. It could be especially useful in aquacultures where molluscides are not allowed.

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Evaluation of three comon tools in estimating *Biomphalaria alexandrina* populations in irrigation ditches.

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Evaluation of three common tools in estimating *Biomphalaria alexandrina* populations in irrigation ditches

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Three snail sampling tools, the dip net, the drag scoop and the modified Ekman grab, were compared under standardized conditions to estimate numbers of *Biomphalaria alexandrina* snails of different sizes in narrow irrigation ditches.

The grab is impractical for general use in the field. It is cumbersome and time consuming, however, it serves as a good reference for comparison of the other methods. The dip net and the drag scoop are simple to manipulate and collect snails, vegetation etc. in a strip of water between the centre and the bank of the ditch. The drag scoop is more efficient in collecting snails than the dip net and gives a better representation of the real density and size distribution of the snails.

Three equations are presented which allow the snail density per m^3 of habitat to be estimated.

Key Words : Snail sampling tools, *Biomphalaria alexandrina*, snail population density.

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In the study of schistosomiasis it is essential to have good means for analysis of snail populations in order to interpret transmission dynamics and to plan effective focal control methodologies. The development of a protocol for snail sampling and selection of the most suitable tools for the job are key issues which must be studied. Several methods have been used by various investigators depending on the objectives of their work. Hairston et al.(1958) evaluated techniques used in estimation of snail populations based on available information without testing them objectively in the field. They concluded that the choice of a sampling method would be determined by the objectives of the study, circumstances under which the work would be done, the nature of the habitat, facilities available and such like. They emphasized that, whatever method was chosen, the worker should evaluate the technique and methodology objectively.

Subsequently Jobin (1970) evaluated an Emery dredge (modified Ekman grab) as a sampling tool for studying snail population dynamics in farm ponds and estimated its recovery rate using scattered coloured buttons.

In the present study an evaluation has been made of three widely used snail sampling tools, the dip net (El Gindy 1966, Jobin 1970, Demian and Kamel 1972), the drag scoop (Dazo et al. 1966, Williams 1970 and Madsen et al. 1987, •), and a modified Ekman dredge (Shiff 1964, Dazo et al. 1966, Jobin, 1970, Boerger 1975, Aboul Ela and Khalil 1989). The efficacy of these tools were studied under simulated natural field conditions typical of those constituting the major portion of the irrigation system of Egypt and several other African countries.

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The evaluation included comparison of convenience of use, recovery rate of snails of different sizes and time involved in collection. The results will help in development of sampling protocols which are more efficient than used in the past.

MATERIAL AND METHODS

The study was conducted in the Snail Research Station of the Theodor Bilharz Research Institute, near Kanater, Qualyubiya Governorate, Egypt. The facility consists of 18 parallel ditches 30 m long, 1.85 m wide narrowing to 1.3 m at water level and 0.5 m wide at the bottom. With inflow and outflow of water constant, the depth was 0.5 m. The ditches were left under natural conditions to establish a normal growth of aquatic vegetation, and each was divided into three 10 m sections with snail proof partitions. The ditches were supplied with underground water, the volume per section was 4.5 m^3.

The snail sampling tools evaluated were the standard dip net, the drag scoop and the modified Ekman dredge, the first two tools were described by Demian and Kamel (1972) and Williams (1970) respectively . They were of the same width, 0.25 m and covered with 10 mesh/cm wire screening. The handles were aluminun tubes, each of 2 m long and the apparatus were made of steel painted to prevent rust. The modified dredge was described by Shiff (1964) and Williams (1970). It was further modified to suit sampling in small ditches. The height of the box was increased to 0.5 m and the four sides to 0.33 m each. The dredge was equipped with a 1.25 m handle of galvanized tube containing a central rod which could be depressed to operate the closing mechanism. The top of

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the grab and two opposite sides were made of wire screening (10 mesh/cm). During operation the dredge was pushed several cm into the mud bottom to collect snails which may be buried in the mud besides capturing floating snails along the water column through which it passed.

The tools were evaluated using four snail densities of Biomphalaria alexandrina, 1000, 2000, 4000 and 8000 snails per ditch section. Size structure of the various snail populations are given in Table 2. Size groups used were small (2-3 mm diam), medium (4-8 mm) and large (9-13 mm). Two replicates of each density were sampled by each tool, therefore 24 ditch sections were used.

The snails used in this study were collected from major drains in Giza Governorate. They were sorted in the laboratory into appropriate size groups, taken to the field and distributed in each ditch section to give the various densities and size distribution required. They were then left for three hours to distribute naturally prior to testing the tools. Each tool was operated in eight sections, twice for each snail density. Sampling was carried out in each section at 20 sites, 0.5 m apart in the case of the dip net and scoop, and at 12 sites in 0.75 m apart the case of the dredge. The dip net was used by passing it smoothly through the water from the edge to the centre of the ditch while the scoop was passed in the opposite direction from the centre of the ditch towards the bank. While the dip net samples floating vegetation, the scoop samples both floating and bottom vegetation/habitat. The time taken to collect, sort and process each sample was recorded.

The sampling processes with the various tools were carried out by the same investigators.

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RESULTS

From the results (Tables 1 and 2) it appears that the main advantage of the dip net is that it requires less effort and a much shorter period for obtaining and recording the number of snails in a sample than the other tools. This is about 15.3% of the time taken by the scoop and 6% of the dredge when the density of snails is 1000/ ditch section . However, it increases with increasing snail density, but always remaining less than time taken with the other tools.

The results also indicate that, for each population density, the dip net and the dredge obtains almost the same number of snails per pass, but both are less than that obtained by the scoop. The mean number of snails obtained by the scoop from the different population densities was 1.5-1.8 times that collected by the other tools.

The recovery rate of snails from the various population densities varies with the three tools in the different densities. However, the most consistent results come from use of the scoop. This apparatus appears to be the most efficient in representing the real population densities. The recovery rates are almost equal in the case of the dip net and dredge, 0.28 ± 0.085 and 0.36 ± 0.124 (95% CL) respectively, whereas this rate reaches 0.4 ± 0.064 in the case of the scoop(Table 1).

When the recovery of different size snails is determined (Table 1.), all three tools represent closely the actual structure of the snail population, however the Ekman dredge is closest to the real structure of the population, and appears to become more accurate with increasing population densities.

Finally it has been possible to deduce an equation for each sampling tool by which estimate of the snail density per m^3 in a water channel similar to those used

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in these experiments can be obtained. These are:

Dip net	$Y = -31 + 71X$	$(r = 0,9655)$
Drag scoop	$Y = -96 + 52X$	$(r = 0,9940)$
Ekman Dredge	$T = -177 + 95X$	$(r = 0,9859)$

where Y= snail density per m^3

X= mean number of snails/ pass

r= correlation coefficient.

DISCUSSION

An efficient snail sampling technique should have a number of features such as ease of operation and handling, suitability for the habitat, an acceptable degree of accuracy in collecting snails and a good representation of the size structure of the snail population. The latter feature is important for estimating certain parameters as reproduction, mortality and growth of snails. The present study has focused on evaluating three commonly used snail sampling tools, the dip net, the scoop and the Ekman dredge for the above mentioned features. It was conducted under natural conditions using populations of live *Biomphalaria alexandrina* with known densities and size structure in similar ditches. The methods used are more natural and realistic than previous trials to study the efficacy of snail sampling tools (Jobin, 1970) when coloured buttons were the target for analysis after being scattered at random in a farm pond.

Clearly the modified Ekman dredge is too cumbersome and time consuming for use in normal snail sampling activities. It does not score well in comparison with the other tools, however it served as a useful means for comparison. The dip net and scoop are easier to handle, and although they sample different parts of the aquatic environment, both yield good estimates of the snail population in numbers and size structure. The scoop,

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because of its basic design has some extra advantages over the dip net. It is more efficient in collecting floating specimens and snails resting on or buried under the bottom mud of the ditches. It is proved to be a convenient snail sampling tool for estimating B. alexandrina population densities, and measuring the size structure of the snail populations.

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Table 1 Evaluation of the dip net, drag scoop and modified Ekman grab as sampling tools of *Biomphalaria alexandrina* from irrigation ditches

Snail density/ ditch section (No./m3)	Dip net *				Drag Scoop *				Ekman grab **			
	1000 (222/m3)	2000 (444/m3)	4000 (888/m3)	8000 (1777/m3)	1000 (222/m3)	2000 (444/m3)	4000 (888/m3)	8000 (1777/m3)	1000 (222/m3)	2000 (444/m3)	4000 (888/m3)	8000 (1777/m3)
Mean No. of snails/sample (± S.D.)	3.3 (±6.3)	9.9 (±10.0)	10 (±9.7)	25.3 (±24.7)	5.1 (±5.0)	12.2 (±10.6)	17.5 (±12.8)	35.5 (±15.7)	3.2 (±2.8)	8.3 (±7.1)	10.8 (±10.7)	20.25 (±17.9)
Mean No. of snails/m3 (± S.D.)	58.9 (±59.18)	178.1 (±199.3)	179 (±178.7)	451.6 (±399.2)	92.4 (±98.79)	217.8 (±204.7)	314 (±243.6)	633.6 (±420.2)	88.98 (±76.65)	224.1 (±234.1)	293.9 (±350.3)	375 (±220.6)
Mean time/ sample (secs) (± S.D.)	9.4 (±7.2)	14.4 (±13.7)	22.7 (±3.81)	36.2 (±7.9)	61.4 (±12.2)	84.7 (±19.7)	92.6 (±21.3)	132.4 (±46.7)	156 (±72.1)	176.8 (±98.3)	181.7 (±87.2)	231.5 (±119.5)
Mean time/m3 (min) (± S.D.)	2.8 (±1.55)	4.29 (±4.17)	6.77 (±0.94)	10.78 (±2.3)	18.27 (±4.64)	25.2 (±7.27)	27.5 (±8.45)	39.45 (±12.29)	70.3 (±24.16)	79.5 (±52.3)	81.9 (±31.7)	71.4 (±38.5)
Recovery rate% (± S.D.)	26.5 (±10.2)	40.1 (±8.31)	20.2 (±4.7)	25.6 (±12.7)	41.6 (±6.77)	49 (±18.2)	35.5 (±9.3)	35.6 (±14.3)	40.8 (±7.2)	50.4 (±4.3)	33.1 (±11.8)	21.1 (±4.3)
Mean recovery rate of all densities (%) (± S.D.)	28.1 (±8.5)				40.4 (±6.4)				36.3 (±12.4)			

* Number of samples = 40

** Number of samples = 24

Table 2 Evaluation of the dip net, drag scoop and modified Ekman grab in representing size groups of *Biomphalaria alexandrina* snail populations.

Snail density/ ditch section	1000			2000			4000			8000		
	S	M	L	S	M	L	S	M	L	S	M	L
Real size structure %	5	70	25	7.5	67.5	25	7.5	67.5	25	10	27.5	62.5
Size structure by dip net % (± S.D.)	13.0 (±2.3)	60.0 (±2.5)	27.0 (±0.2)	21.0 (±4.9)	62.0 (±10.3)	17.0 (±5.2)	13.0 (±5.3)	77.0 (±4.5)	10.0 (±0.8)	22.80 (±28.2)	9.88 (±11.7)	67.32 (±32.31)
Size structure by drag scoop% (± S.D.)	15.0 (±3.7)	68.0 (±3.2)	17.0 (±7.0)	18.0 (±0.6)	64.0 (±0.1)	18.0 (±0.8)	7.0 (±3.4)	83.0 (±2.4)	10.0 (±1.1)	19.47 (±28.2)	24.92 (±21.8)	55.61 (±29.1)
Size structure by Ekman grab% (± S.D.)	23.0 (±2.8)	53.0 (±3.1)	24.0 (±0.4)	20.0 (±4.9)	56.0 (±0.2)	24.0 (±5.2)	7.0 (±6.1)	65.0 (±22.7)	28.0 (±16.5)	13.2 (±22.1)	31.7 (±15.8)	55.1 (±32.7)

S : Small snails (shell diameter = 2-3 mm)

M : Medium snails (shell diameter = 4-8 mm)

L : Large snails (shell diameter = 9-13 mm)

Ecology of *Biomphalaria alexandrina* the snail vector of *Schistosoma mansoni* in Egypt.

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Ecology of Biomphalaria alexandrina the snail vector of Schistosoma mansoni in Egypt

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ABSTRACT

The patterns of spreading and seasonal variation of population density of Biomphalaria alexandrina, the snail vector of Schistosoma mansoni in Egypt, in correlation with some environmental parameters were studied for 1988-1990 in four irrigation canals in Giza and Qalyoubiya Governorates. Results show that the spreading patterns of snails along canals are changeable because of water current and irrigation activities. The snail population density showed two peaks, different in height, in April - May and November - December separated by two bottoms following the Winter Closure and during the hot summer season. The height of the peaks appear to be dependant on the extent of the "Winter Closure" and the prevailing water temperature.

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Two snail sampling tools, the dip-net and drag scoop, were used concurrently in this study. No considerable difference in sampling efficacy was recognized between these tools in the case of Biomphalaria. However, the dip-net appears to be more efficient in sampling other pulmonates while the drag scoop is more efficient in sampling prosobranchs. It is also realized that double sampling of 50% of sampling sites the next day should be satisfactory as a quality control system in snail population studies.

INTRODUCTION

Several national programs of schistosomiasis control based on mass chemotherapy and "focal" mollusciciding were conducted in the last decade in Egypt with variable degrees of success. Efficient "focal" mollusciciding should depend on thorough knowledge of schistosomiasis transmission and on clear indentification of the "transmission focus" in the irrigation system.

The present paper on the ecology of Biomphalaria alexandrina, the snail vector of Schistosoma mansoni, is one in a series of studies conducted by the present authors on schistosomiasis transmission in water courses in Egypt. This study was carried out in four irrigation canals in Qalyoubiya and Giza Governorates and includes distribution of snails along the canals and seasonal variation in snail populations in correlation with certain environmental components of their habitats.

Previous studies of the bionomics of snail intermediate hosts of human schistosomes in Egypt and Middle East are found in the works of El Gindy (1957, 1962), Malek (1958 a,b), Vander Schalie (1958), Watson (1958), Dazo et al, (1966), El Emam and Roushdy (1981) and Madsen et al, (1988).

MATERIAL AND METHODS

Four irrigation canals, namely El Khassa and Radwan canals (Giza Governorate), Sendebis and Sanafeer canals (Qalyoubiya Governorate) were chosen for this study (Table 1). These are typical for secondary and tertiary canals of the irrigation scheme in schistosomiasis area in Egypt. They were selected depending on being infested variably with Biomphalaria alexandrina (Ehrenberg) and not been molluscicided since years. El Khassa canal (a secondary canal) has a relatively low population density of Biomphalaria and a moderate density of aquatic plants. Radwan canal (a tertiary canal) is characterized by a much higher population density of Biomphalaria and dense aquatic vegetation. Sendebis and Sanafeer canals (secondary and tertiary canals respectively) contain moderate densities of Biomphalaria.

Snail populations in these canals were sampled regularly at monthly intervals for one year. Two snail sampling tools, namely the drag scoop (WHO, 1965) and dip-net (Demian and Kamel, 1972)

Table 1 Data of studied canals

Name of canal	Governorate	Name of nearest village	Type of canal	Examined length (m)	Width (cm)	Year of study	Collected Biomphalaria during study
El-Khassa	Giza	Manshiyet Radwan	Secondary	325	300	1988	1244
Radwan	Giza	Manshiyet Radwan	Tertiary	500	125	1988	21530
Sendebis	Qalyoubiya	Sendebis	Secondary	500	225	1988-89	1841
Sanafeer	Qalyoubiya	Sanafeer	Tertiary	300	75	1988-89	3874

were used concurrently. The scoop is 100 cm long, 33 cm wide and 25 cm deep, to collect snails in a strip of water from the mid-line of the water course to water edge at the canal bank. The dip-net is 33 cm long, 33 cm wide and 8 cm deep to collect snails from the bank to the mid-line of the canal. Snail sampling was performed by taking two passes with each tool adjacent to each other in each sampling site. The sampling sites were taken each 25 m. Using two sampling tools should provide better representing data of snail population and further more, allows for comparison of the efficacy of these sampling tools. Snails collected were brought to the laboratory in plastic bags where they were sorted, counted and then replaced the next day to their original sites in canals. Quality control depending on double sampling of snails using the same technique was performed the next day in Sendebis and Sanafeer canals, covering 50% of the sampling sites.

Certain physicochemical parameters namely water depth, temperature, pH and conductivity were measured monthly in each canal. Benthos and aquatic vegetation associated with B. alexandrina were identified and recorded.

RESULTS

1. Distribution of Biomphalaria snails along canals :

Data obtained in this study clearly show that monthly patterns of snail distribution along the present irrigation canals are not

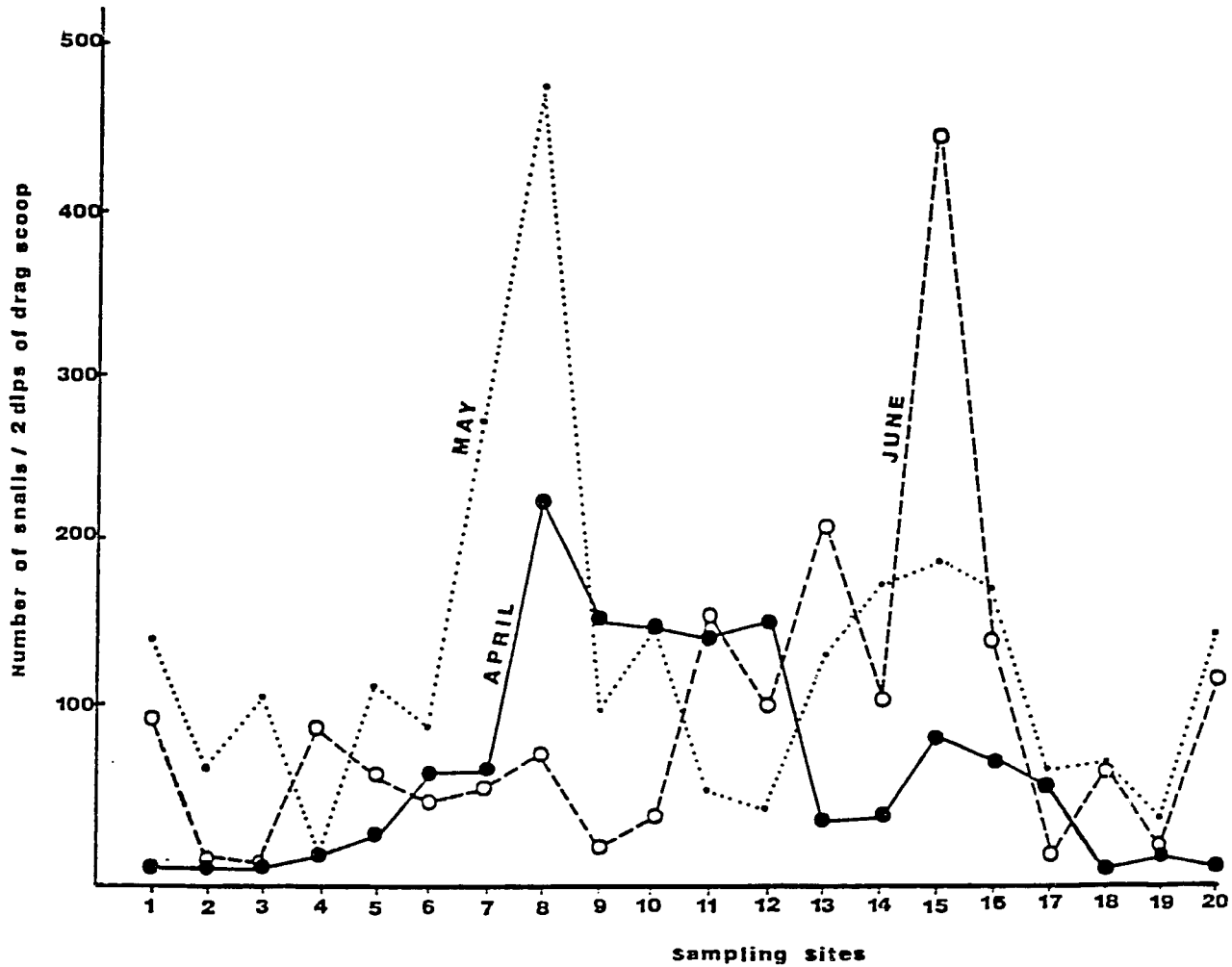

Fig. 1 Distribution patterns of Biomphalaria alexandrina along 500 m of Radwan canal in April, May and June 1988

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exactly similar. Thus, relative densities of snails at various sampling sites change considerably in the same canal from one month to another. For example, in Radwan Canal (Fig. 1), the highest population density appeared in April and May 1988 at site 8, while in June, it was found at site 15.

2. Seasonal Variation in Snail Population :

Two peaks of Biomphalaria population were recognized in the four irrigation canals studied, one major peak and another minor one. In El Khassa and Radwan canals (Figs. 2 & 3), the major peak was found in March - April (1988) while the minor one in December. In that year, the Winter Closure was short and incomplete and the canals did not dry up and consequently snail populations continued to survive and increase forming high peaks early in spring. With the onset of the hot summer time, the snail population decreased gradually to a bottom but rised again in autumn forming a minor peak in December. In Sendebis and Sanafeer canals (Figs. 4 & 5), the major peak appeared in December (1988) while the minor one in March - April (1989). In 1989, the Winter Closure was complete for about 6 weeks in these canals leading to almost drying up of the canal and consequently causing a severe drop in snail populations. After the Winter Closure, the surviving snails laid eggs and the population developed a low peak in "March - April".

3. Ecological Data:

The main physicochemical data of snail habitats were collected

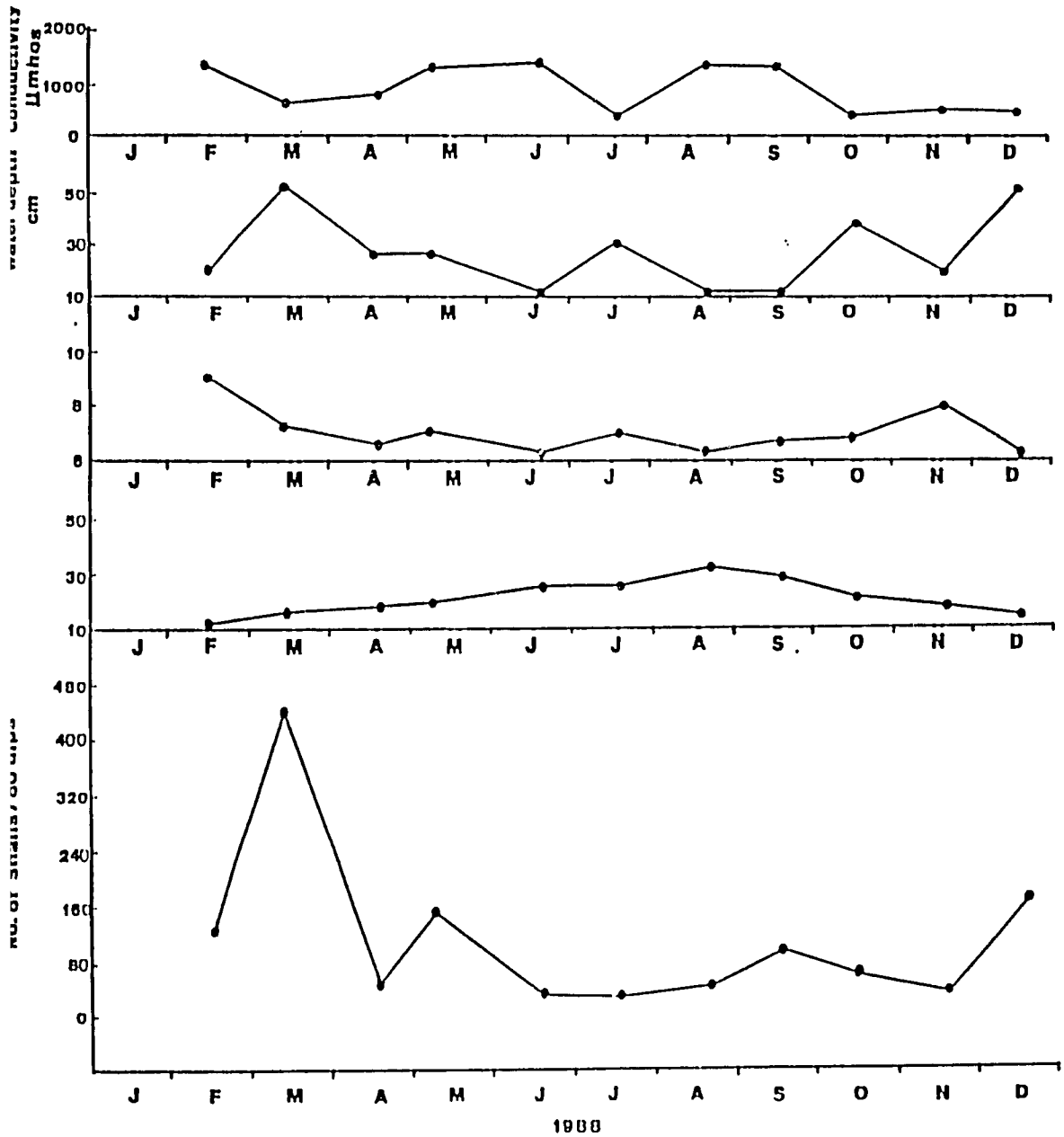

Fig. 2 Seasonal variation in Biomphalaria alexandrina population in El Khassa canal during 1988 and the associated water temperature, pH, depth and conductivity

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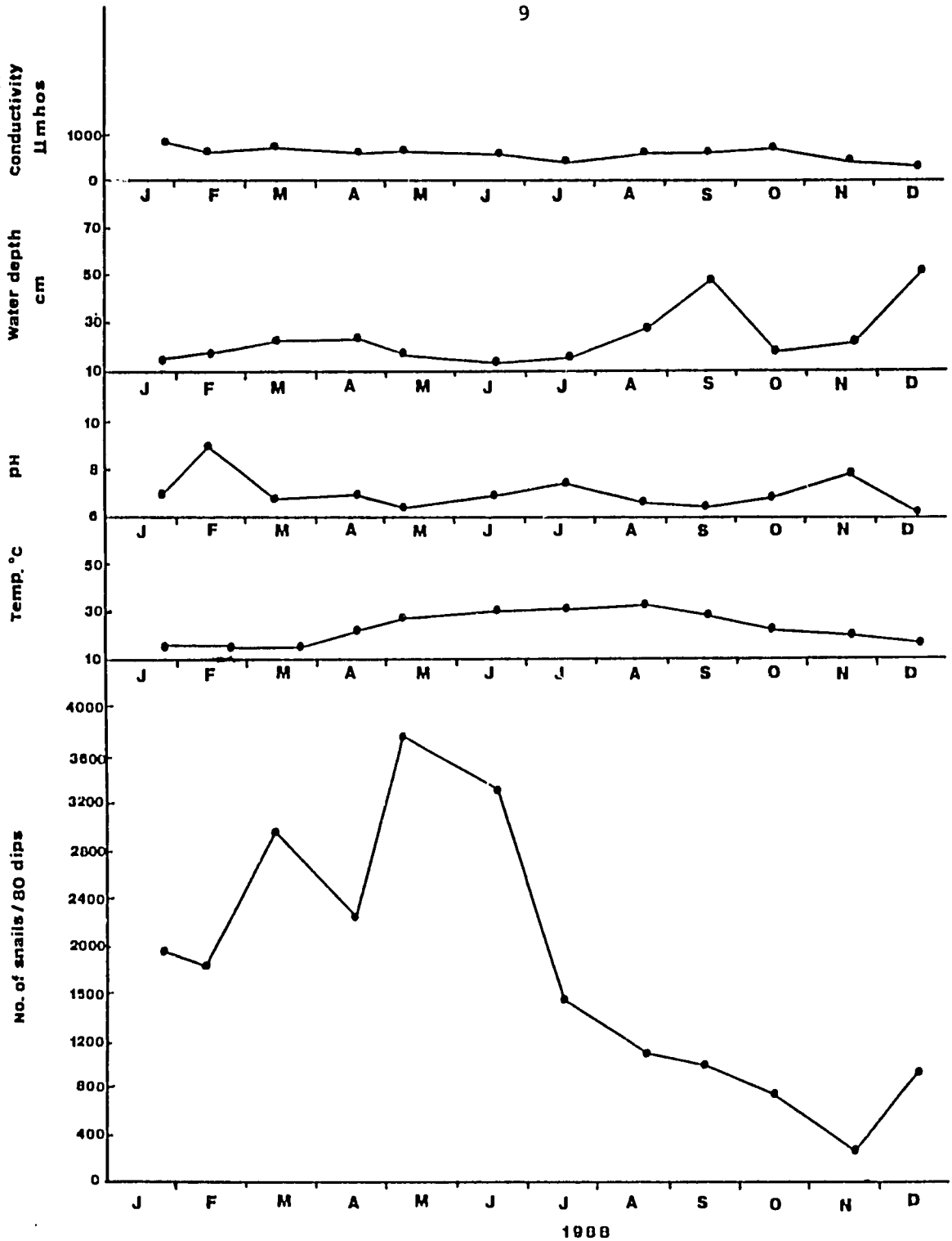

Fig. 3 Seasonal variation in Biomphalaria alexandrina population in Radwan canal during 1988 and the associated water temperature, pH, depth and conductivity

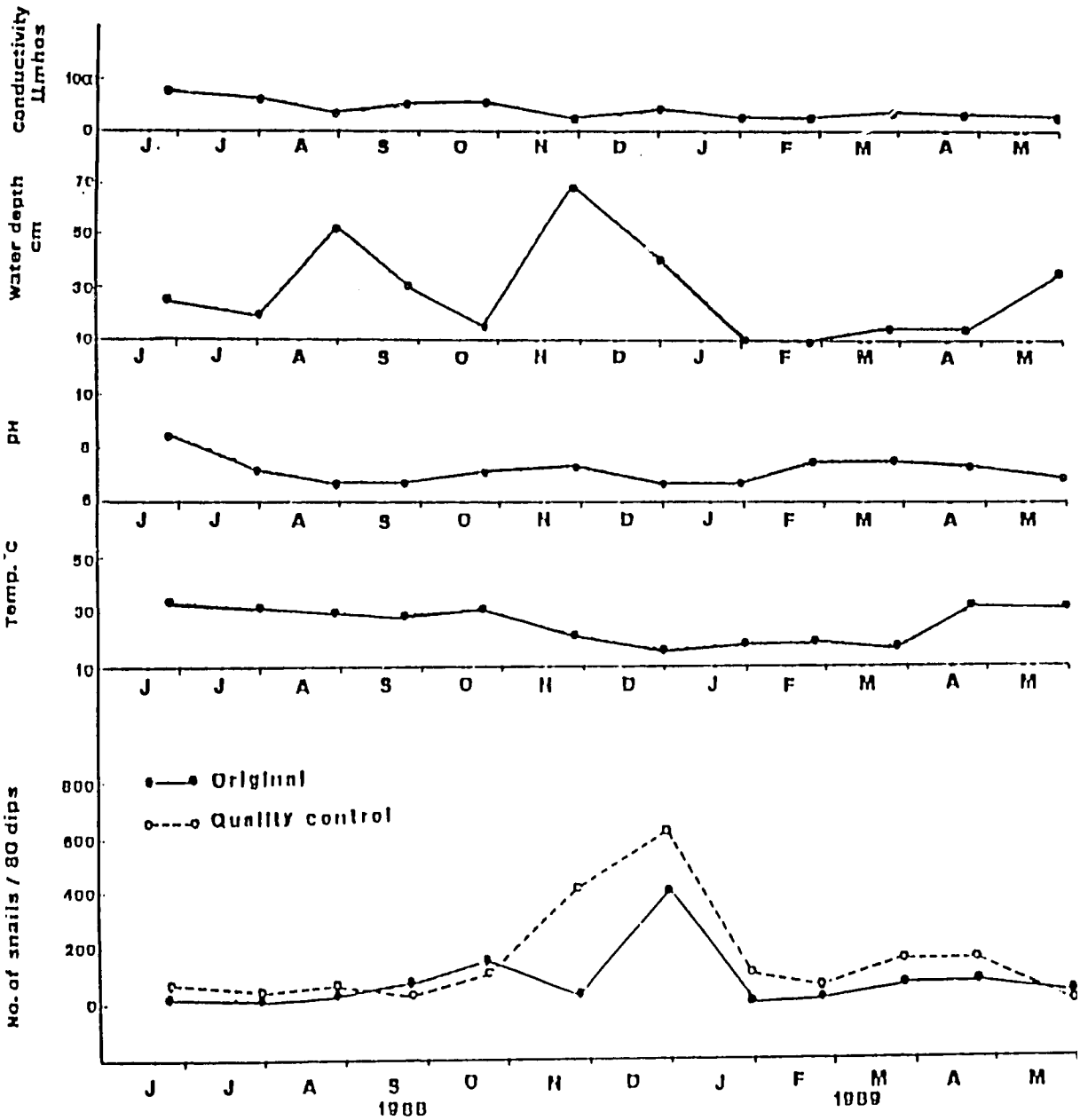

Fig. 4 Seasonal variation in Biomphalaria alexandrina population in Sendebis canal during 1988-1989 and the associated water temperature, pH, depth and conductivity

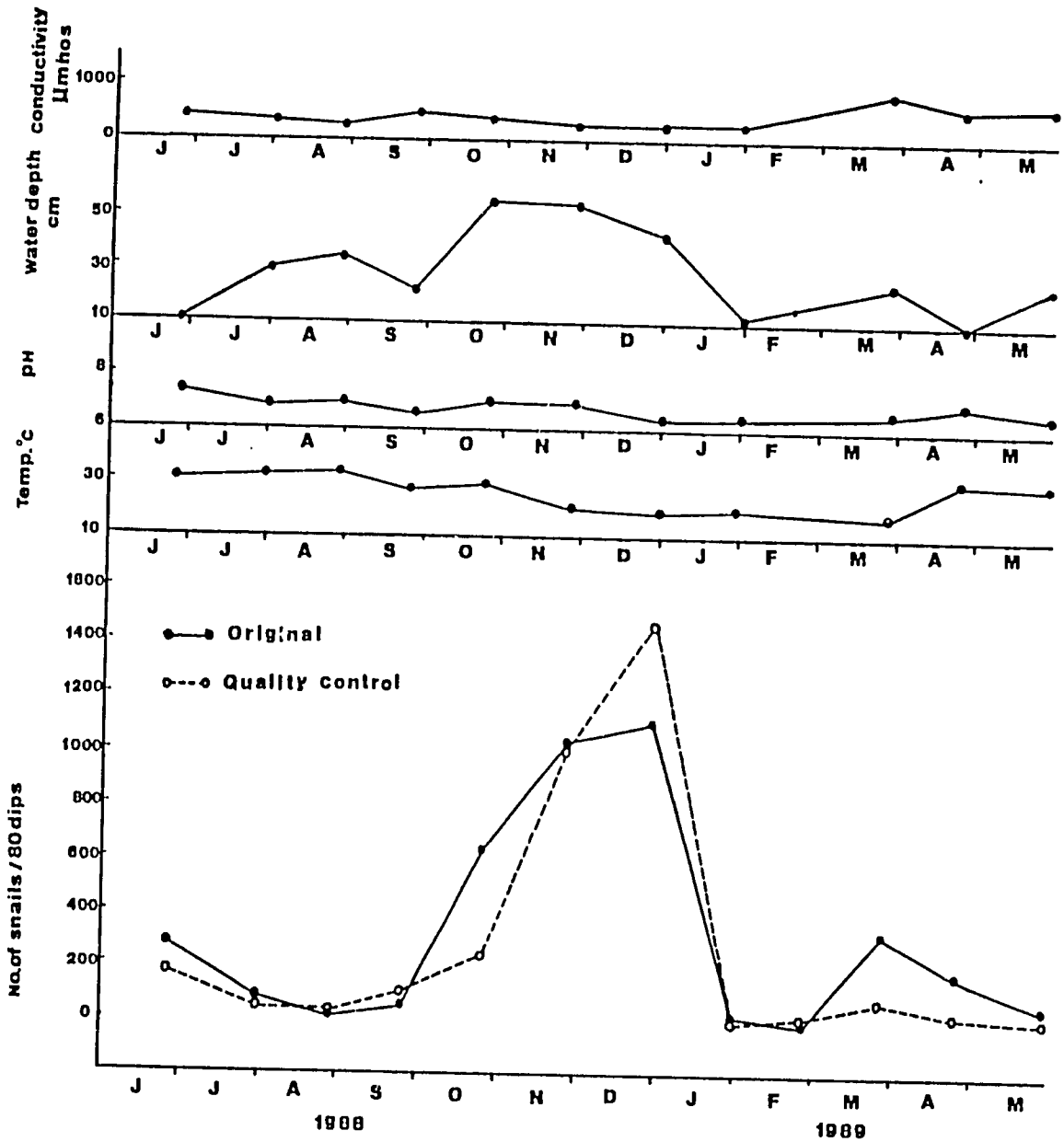

Fig. 5 Seasonal variation in Biomphalaria alexandrina population in Sanafeer canal during 1988-1989 and the associated water temperature, pH, depth and conductivity

in the present four canals throughout one year and are presented in Figs. 2-5. Except during the Winter Closure, the water depth in canals is not linked to seasonality but fluctuates almost regurlarly depending on the irrigation schedules. In January and February, the water level comes down in canals as water is prevented from them for clearing of vegetation (Winter Closure). The canals may dry up or remain wet containing a thin layer of water depending on the extent of this process. This has important reflections on other parameters such as water pH, conductivity and temperature.

The general pattern of water temperature was almost the same in the four canals studied. The mean minimal temperature was ranging from 10°C - 12°C in the cold season (December-March) while in the warm season which extends for six months (April-September) the mean maximum water temperature ranges between 30°C-32°C. The pH value, although changing from one month to an other, was generally around 7 throughout the whole year. However, it increased to 8.8 in February and November 1988 in El Khassa and Radwan Canals. Water conductivity was generally higher in El Khassa than other canals. No direct correlation could be assessed between water pH and conductivity on one side and snail population on the other side.

Several snail species are associated with B. alexandrina in its habitats (Table 2), such as the pulmonates Physa acuta, Lymnaea caillaudi and Planorbis Planorbis and the prosobranchs Valvata

Table 2 Snails associated with *Biomphalaria alexandrina* in the irrigation canals studied (January 1988 - May 1989)

Snail species	Radwan				El Khassa				Sendebis				Sanafeer			
	Dipnet		Scoop		Dipnet		Scoop		Dipnet		Scoop		Dipnet		Scoop	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
A. Pulmonata																
1. *Biomphalaria alexandrina*	10432	74.3	11099	81.3	255	90.7	231	82.8	605	34.0	1175	61.4	1831	76.9	1755	95.7
2. *Physa acuta*	3305	23.5	2377	17.4	17	6.0	7	2.5	1071	60.2	643	33.6	546	22.9	75	4.1
3. *Lymnaea cailliaudi*	161	1.1	128	0.9	7	2.5	34	12.2	100	5.6	86	4.5	2	0.1	1	0.1
4. *Planorbis planorbis*	38	0.3	1	0.01	1	0.4	1	0.4	1	0.1						
B. Prosobranchia																
1. *Valvata nilotica*	87	0.6	36	0.3					2	0.1	5	0.3				
2. *Lanistes carinatus*					1	0.4					6	0.3				
3. *Cleopatra bulimoides*			2	0.01			4	1.4							1	0.1
4. *Bellamya unicolor*	4	0.02														
5. *Theodoxus niloticus*	9	0.01	3	0.02			2	0.7					2	0.8	1	0.1

niloticus, Lanistes carinatus, Cleopatra bulimoides, Bellamya unicolor and Theodoxus niloticus. Physa acuta constitutes the main snail associated with Biomphalaria especially in Sendebis and Radwan canals. Insect larvae and nymph, adult water bugs (Dolomedes and Lethocerus species), annelids, oligochaetes, leeches, nematodes, frogs and fishes were also found in the studied canals. The main associated water plants were Eichhornia crassipes, Phragmites communis, Lemna gibba, Echinocla species, Ceratophyllum demersum, Paspalum species, Potamogeton nodosus and filamentary algae.

4. SNAIL SAMPLING TOOLS AND QUALITY CONTROL :

In the present snail population studies in natural secondary and tertiary irrigation canals, there appears no significant difference in the number of Biomphalaria obtained on sampling by either the dip-net or the drag scoop. However, the results show that the dip-net and drag scoop differ from each other in their efficacy on sampling various other species of snails. Thus, the dip-net is more efficient in sampling Physa acuta and Planorbis planorbis snails, while the drag scoop is more efficient in sampling bottom feeder snails such as Lanistes carinatus (Table 2). As seen in Figs. 4 & 5, quality control results obtained by double sampling of 50% of sampling sites the next day are almost similar to the original results and show the same pattern of snail population fluctuation. This gives indication that this method should be satisfactory as quality control system in snail

population studies.

DISCUSSION

The present results show that snail distribution along the irrigation canals in Egypt changes considerably with time. This may be attributed to water current and irrigation activities in such canals. This feature emphasizes that information in ecology and transmission dynamics should depend on snail sampling as much as possible, if not the whole length of the studied canal, and not to depend on just a few snail samples.

Patterns of snail population density in the four irrigation canals studied show three main features. These are a drop in snail population following the Winter Closure (January-February), a less decrease in the warm season (June-August) and two unequal peaks. This indicates that the most effective ecological factors on snail population density in irrigation canals may be the water depth, aquatic weed clearance and the dominating water temperature. The effect on snail population caused by the Winter Closure although dramatic, nevertheless, it varies depending on extent and duration of this process. If canals dry up complete for a considerably long period, the following peak of snail population is relatively low. If canals remain wet containing water, the sequence of that is an earlier appearance of a spring high peak of snail population.

Winter Closure is usually accompanied with weed clearance which may be also performed in other times of the year. Both factors have suppressive effect on snail populations which are additive during the Winter Closure. Barlow (1935) reported that clearance of canals from weeds may be utilized as an implement in snail control. El-Emam and Roushdy (1981) stated that Winter Closure and aquatic weed clearance greatly affect the population density of snail vectors.

Snails are known to tolerate low water temperatures rather than high temperatures. Thus, increase of water temperature in summer time harms Biomphalaria populations leading to significant decrease in their densities. Snail egg laying appears to take place in early spring and late autumn when water temperature was 20°C±2°C. Gohar and El-Gindy (1960) found that the favourable water temperature for snail vectors in Egypt is 18°C-22°C while Deschiens (1957) found it to be 22°C-26°C. El-Emam and Roushdy (1981) attributed the decrease in snail populations in summer in certain canals (Menoufiya and Giza governorates) to relative increase of water temperature (32°C and 3[illegible]°C) in that season.

The present study shows that the height and location of B. alexandrina population yearly peaks differ in various canals. This agrees with previous findings by other authors. Thus, Dazo et al (1966) found, in irrigation canals near Alexandria, that Biomphalaria peaks were in November-January and May. El Emam and

Roushdy (1981), in their study in Menoufiya Governorate, found that Biomphalaria populations had major peaks in November-January and May in certain irrigation canals and in September-October in drains. These authors added that in El Saff canal (Giza Governorate), one peak only was observed in Biomphalaria population in May, 1980. In Qalyoubiya governorate, Cline et al (1981) described spring and autumn peaks of Biompahlaria.

Regarding the efficacy of the present tools (dip-net and drag scoop), in snail sampling, the present results obtained from secondary and tertiary natural canals, differ from those previously obtained by Yousif et al (under publication) from certain experimental ditches (150 cm wide at water surface & 50 cm deep). Thus, while in the case of the experimental ditches, the drag scoop was more efficient in sampling of Biomphalaria snails (40% recovery) than the dip net (25%), the two tools proved to be equally efficient in natural canals. This may indicates that the efficacy of various snail sampling tools are affected by the size of the water course studied.

In the present study, the highest conductivity values of canal water were found in El Khassa canal reaching 1400 Umhos in the hot season. El Emam and Roushdy (1981) claimed that snails can tolerate water conductivity as high as 1600 Umhos.

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