

THE HOST-PARASITE RELATIONSHIP BETWEEN THE SAUDI ARABIAN *SCHISTOSOMA MANSONI* AND ITS INTERMEDIATE AND DEFINITIVE HOSTS. 1. *S. MANSONI* AND ITS LOCAL SNAIL HOST *BIOMPHALARIA ARABICA*

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INTRODUCTION

Biomphalaria arabica, the snail intermediate host of *Schistosoma mansoni* in Saudi Arabia occurs in habitats ranging from streams, shallow and deep wells to spring, irrigation canals and ditches in Jawf, Sikaka, Khayber and Najran districts (Azim and Gismann, 1956; Arfaa, 1976; Magzoub and Kasim, 1980). The infection rate in natural populations of *Bi. arabica* ranges from 0.6% to 7.4% (Magzoub and Kasim, 1980), while the prevalence of *S. mansoni* in the human population was as high as 74% in Medina (Arfaa, 1976). Transmission of schistosomiasis in Saudi Arabia is focal, mainly in oases where water resources occur and human settlements are located (Jordan and Webbe, 1982).

Covered by a tropical and subtropical desert, Saudi Arabia experiences extreme climatic conditions, with < 250 mm of mean annual rainfall, hot summers (> 30°C) and cold winters (10° to 20°C) (Thrower, 1970). Cloud cover is absent, relative humidity is low and evaporation rate is high. These harsh climatic conditions might influence, directly or indirectly, the success of larval schistosomes in penetrating host snails and their subsequent development into the next stages at least in certain parts of the year (Chu *et al.*, 1966a). The effect of temperature

and salinity on the penetration and development of *S. mansoni* miracidia in their snail hosts was investigated by DeWitt (1955), Purnell (1966), Upatham (1972), Upatham (1973), Sturrock and Upatham (1973) and Prah and James (1977). Findings from these experiments show inconsistencies in the optimal conditions for *S. mansoni* miracidia in penetrating their hosts which may not be sufficiently explained by mere differences in experimental procedures among authors. Strain differences in schistosomes and host snails originating from different localities with different ecological conditions seem to underlie these inconsistencies (Meulemann, 1972).

Similarly, the effect of *S. mansoni* miracidial dose on the infection and cercarial production in different species of *Biomphalaria* studied by Sturrock and Sturrock (1970) and Chu and Dawood (1970) has been shown to be variable. While Pitchford *et al.*, (1969), Asch (1972), Valle *et al.*, (1973) and Théron (1984) observed peaks of daily cercarial emergence in *Biomphalaria* spp. at different times of the day which could be due to differences in the activity pattern of the final hosts. The understanding of the conditions in which a particular strain of a schistosome is transmitted would require an investigation of its relationship with its local hosts. Thus, the aim of this study was to elucidate the host-parasite relationship between the Saudi Arabian *S. mansoni* and its local snail host *Bi. arabica*.

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MATERIALS AND METHODS

Studies described here were conducted at the Center for Applied Malacology and Entomology, Faculty of Science, Mahidol University between January 1986 and September 1986. *Bi. arabica* and *S. mansoni* were obtained from Saudi Arabia and maintained in the laboratory at the Center.

In the laboratory, *Bi. arabica* were reared in plastic bowls using dechlorinated tap water, aerated by the use of air pumps and fed with fresh lettuce. In addition, tropical fish food was given as a supplement. Feeding was done 3 times a week. Egg masses laid by *Bi. arabica* contained 20 to 25 eggs. Young snails were given diatoms and boiled crushed lettuce. Water in bowls containing snails was changed once a week. Temperature of water was kept between 24° and 26°C in an air-conditioned room.

Four experiments were conducted to investigate the host-parasite relationship between *Bi. arabica* and *S. mansoni*.

(1) The effect of exposure dosage of miracidia on the infection rate and cercarial production of *S. mansoni* in *Bi. arabica*:

Six weeks old *Bi. arabica* (about 6 mm in diameter) reared in the laboratory were divided into groups of 30 snails each. Groups I, II, III and IV snails were exposed individually to 1, 2, 4 and 8 miracidia, respectively. Group V snails served as a control and were not exposed to miracidia. The exposure duration was 1 hour in infection plates using dechlorinated tap water at room temperature (25°C). After exposure, each group of snails was reared separately in bowls as mentioned above. Twenty-eight days post-exposure, snails were screened for shedding by keeping them in small vials and illuminating them with artificial light (40 w electric bulb). This was done once every 2 days until they began shedding cercariae. Snail mortality in all

groups was recorded during the pre-patent period.

After the commencement of cercarial shedding in all experimental groups, the number of shedding snails was recorded. Then in all shedding snails in different groups, the number of cercariae shed per snail was estimated as described by Upatham and Sturrock (1973). That is, each shedding snail was kept in a small plastic vial containing 20 ml of dechlorinated tap water and illuminated with artificial light for 6 h. The snails were removed and the cercarial suspension stirred thoroughly, and three 1 ml aliquots were pipetted out, stained with Lugol's iodine and the cercariae counted under a dissecting microscope. The total number of cercariae shed by a snail was estimated by multiplying the mean number of the three 1 ml samples by the total volume of the cercarial suspension (i.e., 20 ml). This was done once a week for 4 consecutive weeks.

(2) Daily pattern of cercarial emergence in *Bi. arabica* infected with *S. mansoni* from Saudi Arabia:

Two groups of positive snails (10 per group) previously exposed to 4 to 6 miracidia each were used in this experiment. The first group was illuminated with artificial light (40 w electric bulb) for 12 h during day light (7.00 a.m. to 6.00 p.m.) and kept in darkness for the next 12 h during the night (7.00 p.m. to 6.00 a.m.) At the same time, the second group was treated in reverse i.e., kept in darkness for 12 h during the day time (7.00 a.m. to 6.00 p.m.) and illuminated with artificial light during the night time (7.00 p.m. to 6.00 a.m.). Cercarial shedding was observed every 2 h for 24 h a day for 4 days.

The number of cercariae shed was estimated in the same way as described above.

(3) The effect of temperature on the infection rate and cercarial production in *Bi. arabica* exposed to miracidia of *S. mansoni*:

A total of 180 snails, 6 weeks old, were divided into 6 groups of 30. Individual snails in the 6 groups were exposed to 4 to 6 miracidia each at temperature ranging from 10° to 40°C at intervals of 6°C. Temperatures were set as follows: 10°, 16° and 22°C in refrigerators, while 34° and 40°C in water baths. The temperature of 28°C was that of water in a room without air-condition and served as a control.

Four to 6 miracidia were pipetted out and introduced into each chamber of the infection plate and left to acclimatize to the exposure temperature of each group for 2 h before snails were added. Meanwhile, 30 snails of each group were also kept under the exposure temperature for the same period of time as the miracidia. Then, one snail was introduced into each chamber of the infection plate under the exposure temperature for 1 h.

After exposure, snails in each group were transferred into bowls and reared under 25°C until cercarial shedding commenced. Snail mortality was recorded during the prepatent period. The number of shedding snails was noted in each group. Cercarial production per snail in each group was estimated once per week for 1 month employing the method described above.

(4) The effect of salinity on the infection of *Bi. arabica* exposed to miracidia of *S. mansoni*:

One hundred and fifty snails of 6 weeks old were divided into 5 groups of 30 each. Group 1 was exposed to 4 to 6 miracidia using dechlorinated tap water with a salinity of 0.5 mg/l and served as a control. The remaining groups (i.e. II, III, IV and V) were exposed to 4 to 6 miracidia at salinities of 1,500, 3,000, 4,500 and 6,000 mg/l, respectively. Different salinity levels were prepared by dissolving the correct amount of NaCl. Snails and miracidia were left to acclimatize

for 2 h into the exposure salinity separately. Exposure time was 1 h. Thereafter, the snails were removed, washed with dechlorinated tap water and reared in the laboratory as mentioned above. During the pre-patent period, mortality of snails was recorded.

RESULTS

The effects of miracidial load on the infection of and cercarial production in *Bi. arabica* exposed to *S. mansoni* from Saudi Arabia are summarized in Fig. 1. The pre-patent period in the different groups was between 30 and 33 days. Snail survival at day 33 post-infection was not significantly different in the different groups exposed to different numbers of miracidia (Chi-square test, $p > 0.1$). Therefore, mortality in snails during the incubation period was not related to the number of miracidia to which snail was exposed.

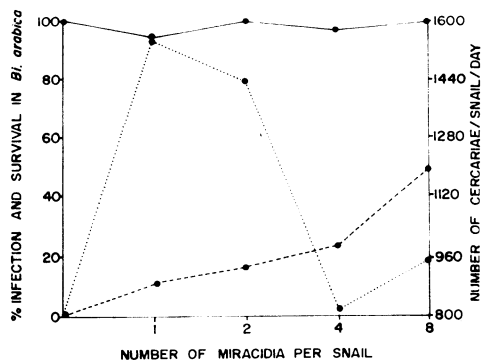


Fig. 1—Effect of miracidial dose on the infection and cercarial production in *Bi. arabica* exposed to miracidia of *S. mansoni* from Saudi Arabia.:
 ● ● = number of cercariae/snail/day, ● ---- ● = % infection rate in snails, ● ——— ● = % survival in snails.

The infection rates in *Bi. arabica* were 10.7, 16.7, 24.1 and 50.0% for snails exposed to 1, 2, 4 and 8 miracidia each, respectively.

A chi-square test showed that there was a significant difference in the infection rates in different snail groups exposed to different numbers of miracidia ($p < 0.01$). Thus, the number of miracidia to which a snail was exposed had an influence on its chance of being infected.

The cercarial production per snail per day was highest (i.e. 1,594 cercariae/snail/day) in *Bi. arabica* exposed to 1 miracidium each and decreased with increasing miracidial load. The Anova test showed that there was a significant difference in the number of cercariae per snail per day in the different miracidial doses ($p < 0.01$). Thus, the number of miracidia to which a snail was exposed had an influence on both the chance of being infected and cercarial production in *Bi. arabica*.

Fig. 2 shows the effects of temperature on the infection of *Bi. arabica* by miracidia of *S. mansoni* from Saudi Arabia. The survival of snails in the different groups was related to

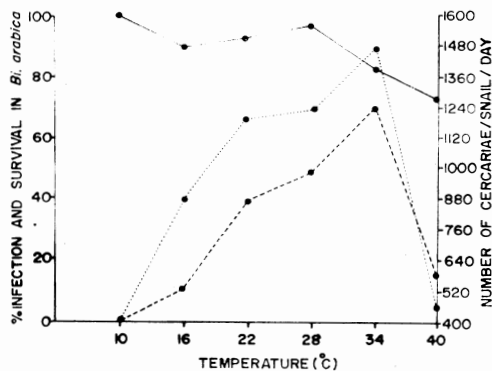


Fig. 2—Effect of temperature on the infection and cercarial production in *Bi. arabica* infected by miracidia of *S. mansoni* from Saudi Arabia. ●.....● = number of cercariae/snail/day; ●-----● = % infection rate in snails; ●————● = % survival in snails.

the exposure temperature ($p < 0.01$) and was lowest in snails exposed to miracidia at a temperature of 40°C. The infection rates in *Bi. arabica* exposed to miracidia at different temperature regimes was highly significantly different ($p < 0.01$). Thus, exposure temperature had an effect on both the mortality and infection rate in snails.

The number of cercariae per snail per day was highest in snails infected by miracidia at a temperature of 34°C. Below and above this temperature, the number of cercariae produced per snail per day declined. The Anova test showed that there was a significant difference in the number of cercariae/snail/day in the different temperature regimes ($p < 0.01$).

Fig. 3 summarizes the results of the effects of salinity on the infection of *Bi. arabica* by miracidia of *S. mansoni* from Saudi Arabia. The mortality in snails in different

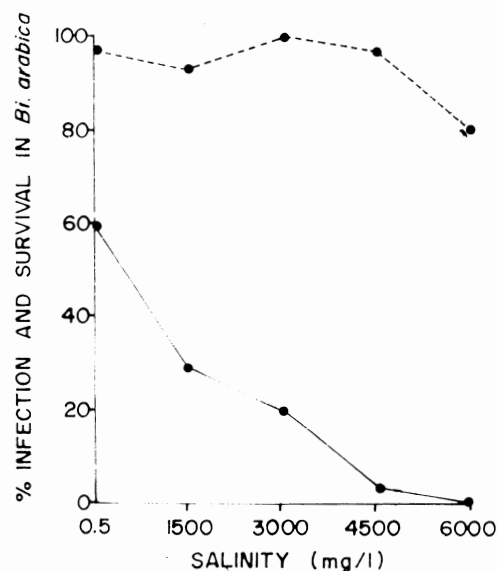


Fig. 3—Effect of salinity on the infection of *Bi. arabica* infected with miracidia of *S. mansoni* from Saudi Arabia. : ●-----● = % survival in snails; ●————● = % infection rate in snails.

salinity levels was not significantly different ($p > 0.5$). Thus, salinity during exposure did not influence the survival of snails during the incubation period. The infection rate in snails exposed to miracidia at salinity of 0.5 mg/l was highest. As the salinity increased, the infection rate in snails decreased up to 3.5% at 4,500 mg/l of NaCl. The Chi-square test showed that the snail infection rates in different salinities was significantly different ($p < 0.01$). Thus, salinity had an influence in the infection of *Bi. arabica* with miracidia of *S. mansoni* from Saudi Arabia.

Fig. 4 (A and B) shows the daily pattern of cercarial emergence from infected *Bi. arabica* snails. The mean number of cercariae

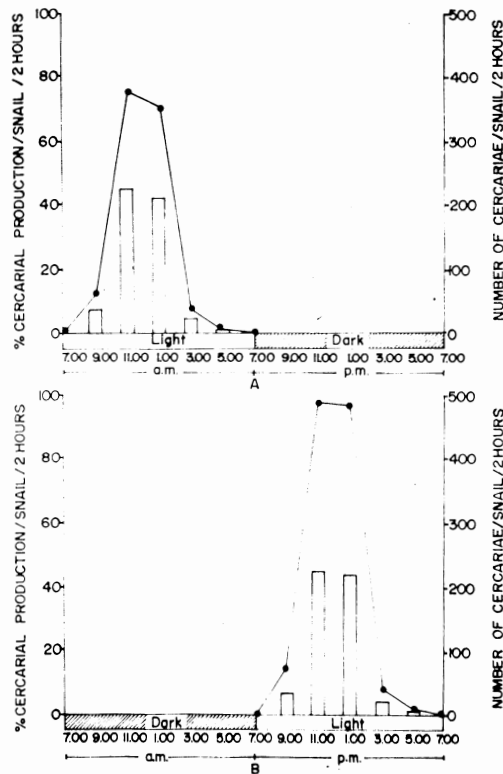


Fig. 4—Showing the cercarial shedding pattern per day in *Bi. arabica* infected with *S. mansoni* miracidia from Saudi Arabia. A = illuminated during day-time, B = illuminated during night-time.

released in 2h period by 10 snails was expressed as a percentage of the total output over 24 h. The result of cercarial shedding from snails exposed to light between 7 a.m. and 7 p.m. (i.e. during day-time) and to darkness between 7 p.m. and 7 a.m. (i.e. during the night-time) are shown in Fig. 4A. It was found that 94.7% of the daily output was released within 6 h, and the peak cercarial emergence occurred between 11 a.m. and 1 p.m. No cercariae were shed during night-time, i.e. during the dark phase. Fig. 4B shows the cercarial shedding pattern during the reverse cycle, i.e. illumination during the night and darkness during the day-time. There was similarity in the pattern of cercarial emergence, in that 95.6% of the cercariae were shed within 6 h. The peak of cercarial emergence occurred between 11 p.m. and 1 a.m. during the illumination phase.

DISCUSSION

Miracidial dose: The number of schistosome miracidia infecting a molluscan intermediate host exerts an influence on the biology of the host and also on their own development to cercariae (Jordan and Webbe, 1982; Jordan *et al.*, 1980, Christensen, 1980). In the present experiment with the Saudi Arabian *S. mansoni* in its local snail *Bi. arabica*, it was observed that the exposure dose of miracidia had an influence on their development in the snail host as determined by the infection rates in snails and the size of cercarial production. It was found that the infection rate in *Bi. arabica* increased with increasing number of miracidia to which the snails were exposed, while the size of cercarial production was largest in snails exposed to 1 miracidium each and decreased as the miracidial dose increased. However, the mortality of snails was negligible in all groups during the prepatent period and was unrelated to the number of infecting miracidia.

A similar trend in infection rates in snails was also reported by Chu *et al.*, (1966b) using *S. haematobium* in *Bulinus truncatus* from Iran. In these experiments, the infection rate in snails increased with increasing number of miracidia to which they were exposed. However, Sturrock and Sturrock (1970) observed that the infection rate and cercarial production increased with increasing miracidial dose in *Bi. glabrata* exposed to 2, 4 and 8 *S. mansoni* miracidia each. Snails exposed to 1 miracidium had the same infection rate as those exposed to 4 miracidia and cercarial production was considerably higher.

Since about 60% of the normal-looking miracidia are unable to produce an infection in susceptible snails (Chernin and Antolics, 1975), it appears that an increase in the number of miracidia to which a snail is exposed would enhance its chance of being infected. It would be expected, therefore, that snails exposed to larger numbers of miracidia would have a higher infection rate than those exposed to smaller numbers. The discrepancy in the size of cercarial production in the present experiment and that observed by Sturrock and Sturrock (1970) can be explained by the lack of interference by other developing miracidia or mother sporocysts in snails exposed to 1 miracidium. Upatham (1976), exposed sentinel snails in field and semi-field conditions in St. Lucia using a novel technique, found that snails (*Bi. glabrata*) infected by 1 miracidium each significantly out-numbered those infected by more than 1 miracidium. Should this be a common phenomenon in *S. mansoni*, then the larger number of cercariae produced by snails infected by 1 miracidium may be an adaptation to compensate for the losses of miracidia which do not produce an infection (Chernin and Antolics, 1975) and, therefore, ensure transmission, since most snails are infected by only 1 miracidium (Upatham, 1976).

Cercarial emergence pattern: It has been generally established that in *S. mansoni* the total number of cercariae per day is released within 5 h, while the peak emergence is reached after about 3 h (Jordan *et al.*, 1980). Previous studies had also shown that daily cercarial emission from infected snails follows a rhythmic pattern with a peak at the time of maximum activity of the definitive hosts (Pitchford *et al.*, 1969; Asch, 1972; Valle *et al.*, 1973; Théron, 1984). The same studies have also shown that the time of the day at which the peak cercarial emergence occurs differs from one endemic area to another, apparently due to variation in the water contact pattern of the vertebrate host in snail habitats. In *Bi. arabica* from Saudi Arabia, cercariae were shed between 9.00 a.m. and 7.00 p.m. with 94.7% of the total production being released within 6 h. The peak cercarial emergence occurred between 11.00 a.m. and 1.00 p.m.

Chu and Dawood (1970) using an Egyptian *S. mansoni* in *Bi. alexandrina* established that the pattern of cercarial emergence in laboratory and naturally infected snails was similar. It follows that our findings with laboratory infected *Bi. arabica* may represent the daily pattern of cercarial emission in nature (Saudi Arabia). This would imply that water contact before 11.00 a.m. poses little risk of infection of the definitive host. Tameim *et al.* (1985) reported a significant reduction of prevalence of *S. mansoni* in a group of canal cleaners in the Gezira irrigation project, Sudan after their working hours were changed so that they left the water at 10.00 a.m. before the peak cercarial emergence occurred at mid-day. Therefore, in parts of Saudi Arabia where *S. mansoni* is endemic, a schedule of activity in water resources such as that adopted in the Gezira irrigation canals would seem indicative for the prevention of schistosomiasis.

Effects of temperature: As pointed out by Prah and James (1977), temperature affects the metabolic processes in the miracidia,

thus directly affecting their degree of activity and hence their survival and infectivity. In the present study with the Saudi Arabian isolate of *S. mansoni* in its local snail *Bi. arabica*, the penetration and subsequent development of miracidia as determined by infection rates and cercarial production appeared to be directly related to water temperature during exposure. The optimum temperature range which obtained high infection rates was 28° to 34°C. Below and above this temperature range, the infection rates declined. There was no infection of snails at temperatures below 16°C.

The studies of DeWitt (1955) on the infectivity of Puerto Rican *S. mansoni* in *Bi. glabrata* showed no infection of snails at 10°C, but above this temperature there was an increase in infection rates as the temperature increased to 35°C. While the Tanzanian *S. mansoni* miracidia studied by Purnell (1966) were able to infect *Bi. sudanica tanganyicensis* at temperatures ranging from 9° to 36°C with infection rates increasing linearly with temperature. Chu *et al.*, (1966a) using the Iranian *S. haematobium* in *Bu. truncatus* observed an increase in infection rates in snails exposed at temperatures ranging from 10° to 30°C, above which there was a decrease. Prah and James (1977) studied the effect of temperature on the initial penetration of *S. mansoni* and *S. haematobium* miracidia in *Bi. pfeifferi* and *Bu. truncatus*, respectively. In both cases, there was no infection of snails below 15°C, thereafter infection rates increased with increasing temperature up to 35°C.

Judging from the findings of DeWitt (1955), Chu *et al.*, (1966a). Upatham (1973) and those of the present experiment, it seems that the Saudi Arabian *S. mansoni* miracidia are more tolerant to higher temperatures and produce appreciable snail infection rates at 40°C. Since high water temperatures of up to 40°C are likely to occur in Saudi Arabia

during summer, they will only reduce but not inhibit the transmission of *S. mansoni*, at least at the level of the miracidia. On the other hand, temperatures below 16°C which occur during winter would seem to inhibit infection of snails by *S. mansoni* miracidia, thus setting in a seasonality of transmission. However, field studies are required to confirm our laboratory findings.

Exposure temperature does not only effect the initial penetration process of miracidia as believed by Prah and James (1977), but also their development into sporocysts (Upatham, 1973) and hence their maturation into cercariae (Jordan *et al.*, 1980). In the present study, the size of cercarial production rose with increasing temperature up to 34°C after which it declined.

There is a slight disagreement with the results of Barbosa (1962) quoted by Jordan *et al.*, (1980) whereby a temperature of 35°C almost completely inhibited cercarial production of *S. mansoni* in *Bi. glabrata*. However, Barbosa was referring to snail maintenance temperature during the pre-patent period as opposed to exposure temperature studied in the present work. All the same, high temperatures in excess of 34°C during exposure affect the miracidia adversely such that their subsequent development in the snail to cercariae is also affected. Although *S. mansoni* miracidia are able to produce appreciable infection rates in *Bi. arabica* at 40°C, cercarial production is extremely low at this temperature.

Effect of salinity: Salinity of water during exposure of snails to schistosome miracidia has been found to influence the penetration process (Upatham, 1972; Chernin and Bower, 1971). In the study reported here with the Saudi Arabian *S. mansoni*, the infection rates in *Bi. arabica* decreased from 58.6% at a salinity of 0.5 mg/l to 3.4% at 4,500 mg/l.

Above this salinity, no infection of snails occurred.

Upatham (1972) investigating the effect of salinity on the infection of *Bi. glabrata* by the St. Lucian *S. mansoni* miracidia observed that the infection rates in snails decreased curvilinearly at increasing levels of NaCl until 4,200 ppm, above which no infection occurred. Chernin and Bower (1971) found that the infectivity of *S. mansoni* miracidium was altered in brackish water with salinity of up to 2.39‰, and above this level it progressively decreased.

It seems that the Saudi Arabian *S. mansoni* miracidia are more tolerant to higher salinities than the St. Lucian one studied by Upatham (1972). This might explain the fact that some schistosomiasis endemic areas in Saudi Arabia (such as Najran and Khayber) are quite close to the sea and may contain water with high salinity levels but transmission seems not to be affected.

SUMMARY

The infectivity of miracidia of the Saudi Arabian isolate of *S. mansoni* in *Bi. arabica* was found to be influenced by such factors as miracidial dose, water temperature and salinity.

The pre-patent period of *S. mansoni* in *Bi. arabica* was 30 to 33 days. Miracidial dose had no effect on the mortality of snails during the pre-patent period. The infection rate increased as the miracidial dose was increased. However, cercarial production was highest in snails exposed to 1 miracidium each and decreased as the miracidial dose was increased.

Water temperature during exposure had an influence on the mortality, infection rate and cercarial production in *Bi. arabica* exposed to *S. mansoni* miracidia. The infection rate was highest in snails exposed at 28° and 34°C.

No infection of *Bi. arabica* occurred at the temperature of 10°C. The number of cercariae per snail per day was highest in snails exposed to miracidia at 34°C.

It was demonstrated that salinity had an influence on the infection of *Bi. arabica* with miracidia of *S. mansoni*. The infection rate in snails decreased as the salinity increased up to 4,500 mg/l, above which no infection occurred.

The daily pattern of cercarial emergence was rhythmic, whereby 94.7% of the total daily production was released within 6 h from infected *Bi. arabica*, with a peak between 11 a.m. and 1 p.m.

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