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The Emergence of Schistosome Cercariae from the Snails 3. Combined Effect of Light and Temperature on the Emergence of *Schistosoma mansoni* and *S. haematobium* Cercariae

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Key words: *Schistosoma mansoni, Schistosoma haematobium,* cercaria, emergence, periodicity, circadian rhythm

Introduction

The findings of several workers who have studied the effect of photoperiod inversion on cercarial emergence, suggest that exogenous factors such as light and temperature play an important role in the rhythmic emergence of some species of cercariae from their snail hosts. Giovannola (1936) studied the effect of photoperiod inversion on the emergence of nonschistosome cercariae, Olivier (1951) examined on *Schistosomatium douthitti* cercariae, and Luttermoser (1955), Valle *et al.* (1971), Asch (1972) and Glaudel and Etges (1973) investigated on *Schistosoma mansoni* cercariae. However, to our knowledge, no photoperiod inversion studies have been reported with respect to *S. haematobium* cercariae.

The present investigation was undertaken to determine the combined effect of light and temperature on cercarial emergence and to elucidate the mechanism underlying the diurnal emergence of *S. mansoni* and *S. haematobium* cercariae.

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Materials and Methods

The emergence of a Puerto Rican strain of *S. mansoni* and of a Kenyan strain of *S. haematobium* cercariae from Puerto Rican *Biomphalaria glabrata* and Kenyan *Bulinus globosus* snails, respectively, was studied for 14 days or more under controlled photo- and thermoperiodic conditions.

Snails, individually infected with 5 miracidia of *S. mansoni* or *S. haematobium* were maintained in circulating-water (27–30 C) aquaria under continuous 1,500 lux illumination, so that they were not exposed to a natural light cycle after infection. When they began to release cercariae, several groups of 4 snails each per experiment were transferred to 15 cm diameter petri dishes containing 200 ml of dechlorinated tap water; these dishes were placed into an incubator and changed into new at 8:30 a.m. and again at 8:30 p.m.

During the light period, illumination (1,500 lux) was produced with a 20 W fluorescent lamp (National FL 20S-W); darkness was complete during the dark period.

After removal of snails, cercariae in the old dish were counted by our method (Nojima and Sato, 1978); briefly, the cercariae were formalin-fixed and counted

under a dissecting microscope. If there were too many cercariae to permit exact counting, the total count was estimated from the number of cercariae on some parts of the dish. Counts made at the inception of a photoperiod were regarded as representing the number of cercariae released during the preceding dark period.

In the figures, the average of cercariae released per snail, calculated from double, identical experiments, is shown. Cercarial emergence occurring in the 12 hr period from 8:30 a.m. to 8:30 p.m. was compared with that occurring between 8:30 p.m. and 8:30 a.m. throughout the experiments. When during one of these periods emergence was greater than during the other period, this was recorded as positive rhythmic emergence.

Results

Diurnal emergence under laboratory conditions

Prior to these experiments, the snails were transferred from an aquarium in continuous illumination to dishes in diffused sunlight near window at laboratory (24–26 C, daytime; 22–24 C, night), and maintained for 2 days.

The subsequent emergence of *S. mansoni* and *S. haematobium* cercariae during the 15-day experimental observation period is shown in experiments MD and HD (Fig. 1), respectively. Under natural conditions, cercarial emergence was quite diurnal and photoperiodic in both species.

Photoperiodic emergence at 12-hr light/12-hr dark exposure at a constant water temperature of 25 C

The emergence of *S. mansoni* and *S. haematobium* cercariae during an 18-day observation period is shown in experiments MP and HP (Fig. 2), respectively. In both species, cercarial emergence during the light period was always higher than during the dark period, indicating photoperiodicity under these conditions. Although cercarial emergence of *S. haematobium* was greatly suppressed during the dark period, this was not the case with *S. mansoni*.

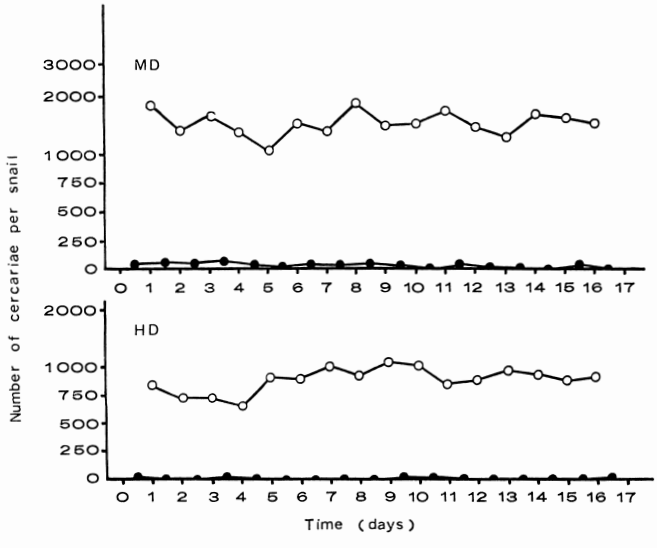

Fig. 1 Diurnal emergence of *S. mansoni* cercariae (MD) from *B. glabrata* and *S. haematobium* cercariae (HD) from *B. globosus* under laboratory conditions.
○ : Cercarial emergence during the daytime (8:30 a.m.—8:30 p.m.)
● : Cercarial emergence during the night-time (8:30 p.m.—8.30 a.m.)

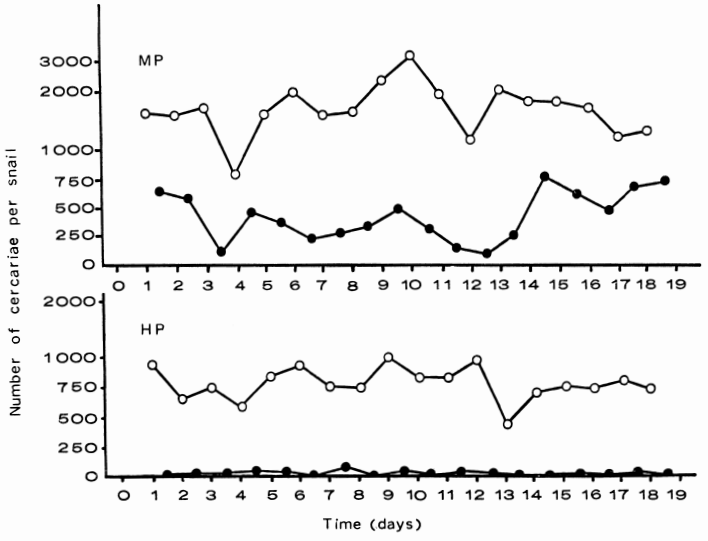

Fig. 2 Photoperiodic emergence of *S. mansoni* cercariae (MP) from *B. glabrata* and *S. haematobium* cercariae (HP) from *B. globosus* exposed to a 12-hr light (1,500 lux)/ 12-hr complete dark cycle at a constant water temperature of 25 C.
○ : Cercarial emergence during the light period (8:30 a.m.—8:30 p.m.)
● : Cercarial emergence during the dark period (8:30 p.m.—8:30 a.m.)

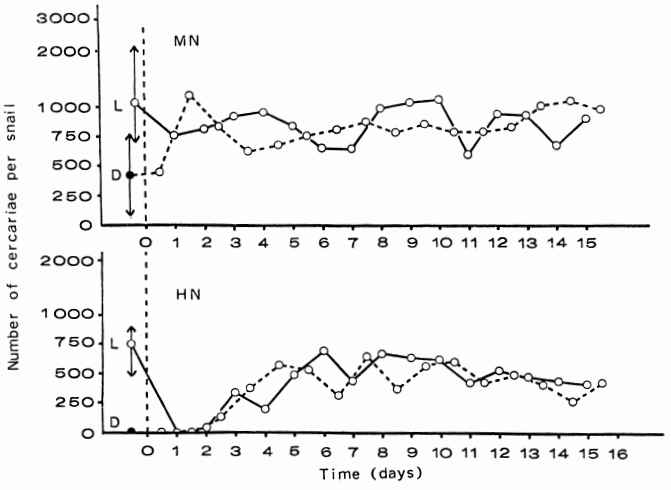

Fig. 3 Nonperiodic emergence of *S. mansoni* cercariae (MN) from *B. glabrate* and *S. haematobium* cercariae (HN) from *B. globosus* exposed to continuous illumination (1,500 lux) at a constant water temperature of 25 C.
—○—: Cercarial emergence from 8:30 a.m. to 8:30 p.m.
--○--: Cercarial emergence from 8:30 p.m. to 8:30 a.m.

These findings indicate that a 12-hr light/ 12-hr dark cycle at constant water temperature induced rhythmic emergence in both cercarial species and that photoperiodicity is more pronounced in *S. haematobium* than in *S. mansoni*.

Nonperiodic emergence at a constant water

temperature of 25 C under conditions of continuous illumination

Prior to these experiments, the snails were maintained for 14 days at a constant water temperature of 25 C and exposed to a 12-hr light/12-hr dark cycle. As indicated to the left of day 0 in Fig. 3, cercarial emergence in both species revealed photoperiodicity.

The subsequent emergence of *S. mansoni* and *S. haematobium* cercariae during the 15-day experimental observation period is shown in experiments MN and HN (Fig. 3), respectively. Under these experimental conditions, cercarial emergence was nonrhythmic in both species and the pre-experimental periodicity of emergence disappeared soon after inception of the experimental continuous light exposure. In *S. haematobium*, cercarial emergence was markedly depressed during the first 2 days of the experiment and recovered gradually thereafter. In *S. mansoni*, no initial suppression of cercarial emergence was noted.

Temperature-induced periodic emergence during continuous light exposure at cyclic temperature variation

Prior to these experiments, the snails were maintained for 14 days at a constant water temperature of 25 C and exposed to continuous illumination. As indicated to the left of day 0 in Figs. 4 and 5, cercarial emergence in both species was nonperiodic during this period.

Two experiments were performed with *S. mansoni*. In experiment MT-1 (Fig. 4), the snails were exposed for 16 days to continuous illumination. During this period, the water temperature was 26.5 C during

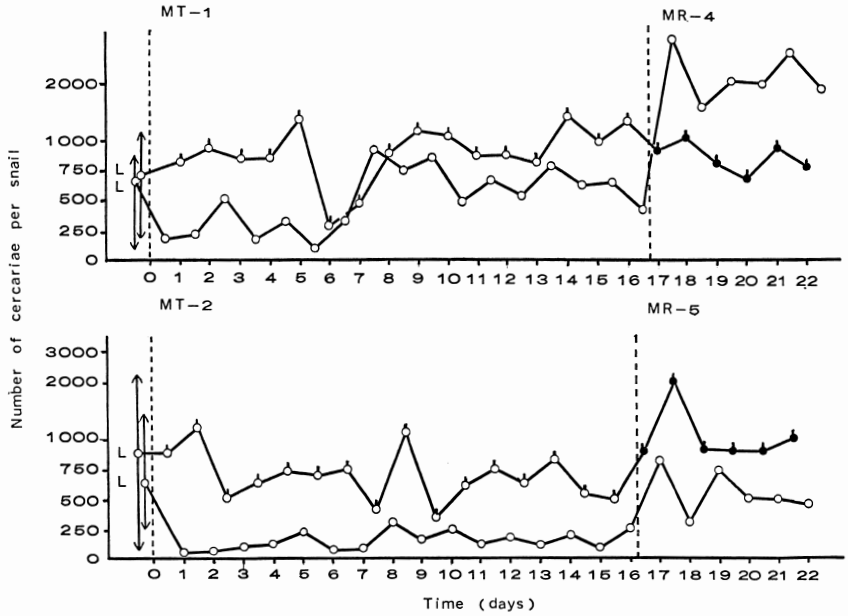

Fig. 4 Thermoperiodic emergence of *S. mansoni* from *B. glabrata* exdosed for 16 days to continuous illumination (1,500 lux) and 12-hr cyclic temperature variations of 3 C (MT-1) and 6 C (MT-2).

○ : 26.5 C (MT-1), 28 C (MT-2), continuous illumination; ● : 26.5 C (MR-4), 28 C (MR-5), dark period

○ : 23.5 C (MT-1), 22 C (MT-2), continuous illumination; ○ : 23.5 C (MR-4), 22 C (MR-5), light period

the hours from 8:30 a.m. to 8:30 p.m. and 23.5 C from 8:30 p.m. to 8:30 a.m., i.e. the temperature difference between the cycles was 3 C.

In experiment MT-2 (Fig. 4), the snails were exposed for 16 days to continuous illumination. During this period, the water temperature was 28 C from 8:30 p.m. to 8:30 a.m. and 22 C from 8:30 a.m. to 8:30 p.m., i.e. the temperature difference between the cycles was 6 C.

As shown in Fig. 4, during the 16-day continuous illumination period, with the exception of days 6, 7 and 8 in experiment MT-1, cercarial emergence of *S. mansoni* was always higher at the higher temperature, indicating thermoperiodicity.

The emergence of *S. haematobium* cercariae during the 15-day observation period following 14-day continuous light exposure at a constant water temperature of 25 C is shown in Fig. 5.

In this experiment (HT-1, Fig. 5), the snails were exposed to continuous illumination for 14 days. During this period, the water temperature was 26.5 C from 8:30 p.m. to 8:30 a.m. and 23.5 C from 8:30 a.m. to 8:30 p.m., i.e. the temperature difference between the cycles was 3 C. As in *S. mansoni*, cercarial emergence in *S. haematobium* was thermoperiodic during this 15-day observation period.

These findings indicate that rhythmic emergence could be induced by temperature cycles in both cercarial species.

Furthermore, in this series of experiments, we examined the effects of introducing reversed cyclic temperatures (low temperature during the light period, high temperature during the dark period) on the established thermoperiodicity of cercarial emergence. Therefore, starting with day 17 (*S. mansoni*) or day 15 (*S. haematobium*), cyclic 12-hr light/12-hr dark periods were introduced.

In experiment MR-4 (Fig. 4), the water temperature was 23.5 C during the light period and 26.5 C during the dark period (3 C difference). In experiment MR-5 (Fig. 4), the water temperature during the light period was 22 C, during the dark period it was 28 C (6 C difference).

In the experiment with *S. haematobium* (Fig. 5), starting with day 15, the snails were exposed to a constant water temperature of 25 C during the succeeding light/dark cycles.

Photoperiodic versus thermoperiodic emergence of cercariae

In these experiments, the effects of reversed cyclic temperatures (low temperature during the light period, high temperature during the dark period) on cercarial emer-

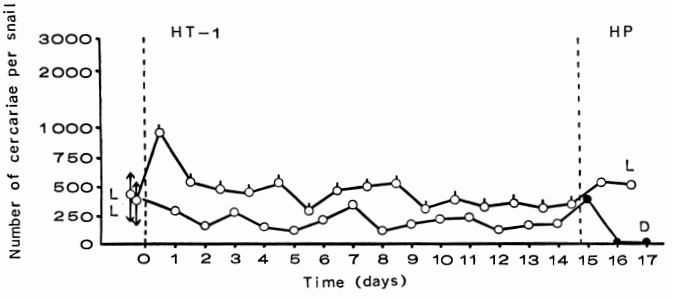

Fig. 5 Thermoperiodic emergence of *S. haematobium* cercariae from *B. globosus* exposed for 14 days to continuous illumination (1,500 lux) and 12-hr cyclic temperature variation of 3 C.

♂ : 26.5 C, continuous illumination (HT-1); ○ : 25 C, light period (HP)
○ : 23.5 C, continuous illumination (HT-1); ● : 25 C, dark period (HP)

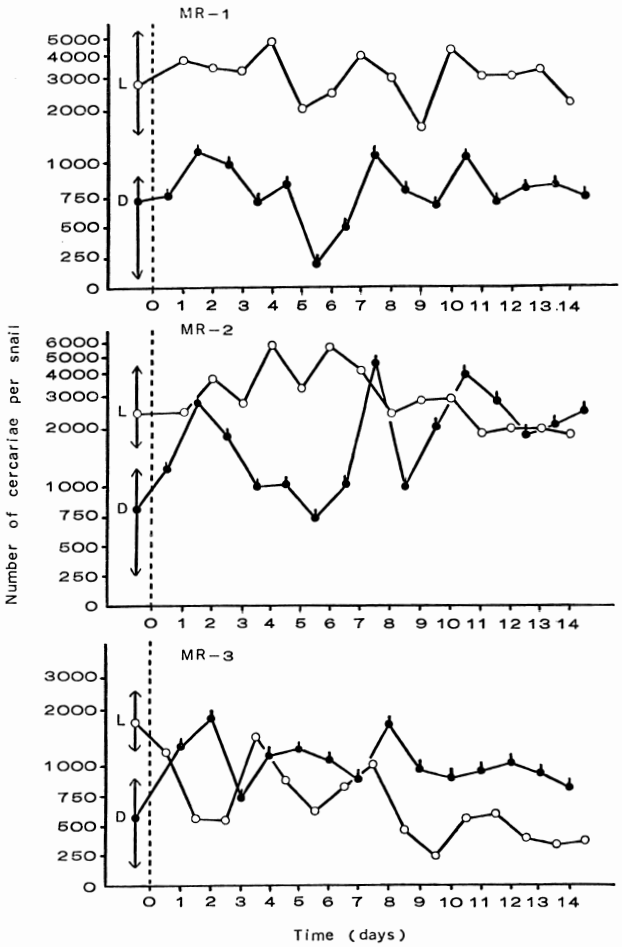

Fig. 6 Photoperiodic (MR-1), nonperiodic (MR-2) and thermoperiodic (MR-3) emergence of *S. mansoni* cercariae from *B. glabrata* exposed for 14 days to 12-hr light (1,500 lux)/12-hr complete dark periods and reversed cyclic temperatures.
● : 26.5 C (MR-1), 27.5 C (MR-2) & 28 C (MR-3), dark period
○ : 23.5 C (MR-1), 22.5 C (MR-2) & 22 C (MR-3), light period

gence were studied.

Prior to the experiments, the snails were maintained for 14 days at a constant water temperature of 25 C and exposed to a 12-hr light/12-hr dark cycle. As indicated to the left of day 0 in Figs. 9 and 10, cercarial emergence in both species was photoperiodic during this period.

In experiments MR-1, MR-2 and MR-3 (Fig. 6), *B. glabrata* were exposed for 14 days to a 12-hr light/12-hr dark cycle. In experiment MR-1, the water temperature during the light period was 23.5 C, and during the dark it was 26.5 C (3 C difference); in MR-2 it was 22.5 C during the light- and 27.5 C during the dark cycle (5 C difference); in MR-3 it was 22 C during the light- and 28 C during the dark cycle (6 C difference).

As shown in Fig. 6, while the pre-experiment photoperiodic emergence of *S. mansoni* persisted under reversed cyclic temperature conditions if the temperature difference between the light- and dark cycle

was 3 C (MR-1), it disappeared when the temperature difference was greater than 3 C (MR-2, MR-3). With the exception of days 3 and 7, thermoperiodicity was clearly manifested in MR-3 where the temperature difference was 6 C.

We ascribe the difference between the number of cercariae released in experiments MR-1 and MR-2 and those released in experiment MR-3 to the size difference of their snail hosts.

In the experiments shown in Fig. 4, upon introducing the light/dark cycle and reversed cyclic temperatures on day 17, *S. mansoni* cercarial emergence from snails of experiment MR-4 (3 C difference) changed from thermoperiodic to photoperiodic, while that from snails of experiment MR-5 (6 C difference) remained thermoperiodic. In the experiment shown in Fig. 5, introducing the 12-hr light/12-hr dark cycle and maintaining the water temperature at 25 C effected a change from thermoperiodicity to photoperiodicity in *S. haematobium* cercarial emergence.

In experiments HR-1, HR-2 and HR-3 (Fig. 7), *B. globosus* were exposed for 14 days to a 12-hr light/12-hr dark cycle. In

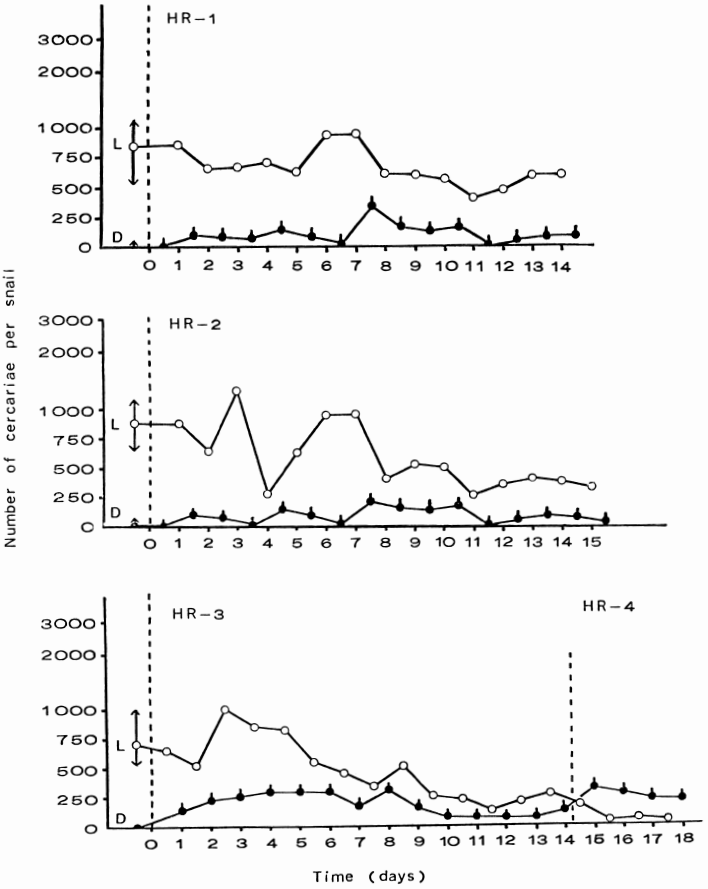

Fig. 7 Photoperiodic (HR-1, HR-2 & HR-3) and thermoperiodic (HR-4) emergence of *S. haematobium* cercariae from *B. globosus* exposed for 14 days to 12-hr light (1,500 lux)/12-hr complete dark periods and reversed cyclic temperature.

● : 26.5 C (HR-1), 27 C (HR-2), 28 C (HR-3) & 29 C (HR-4), dark period

○ : 23.5 C (HR-1), 22 C (HR-2), 22 C (HR-3) & 21 C (HR-4), light period

experiment HR-1, the water temperature during the light period was 23.5 C, during the dark it was 26.5 C (3 C difference); in HR-2 it was 22 C during the light- and 27 C during the dark cycle (5 C difference); in HR-3 it was 22 C during the light- and 28 C during the dark cycle (6 C difference). Furthermore, in experiment HR-3, starting with day 15, the temperature difference between the light/dark cycle was increased further by exposing the snails during the light period to a water temperature of 21 C; during the dark period, the temperature was 29 C, resulting in an 8 C temperature difference (HR-4 in Fig. 7).

As shown in Fig. 7, the pre-experiment photoperiodic emergence of *S. haematobium* persisted under conditions of reversed cyclic temperatures if the temperature difference between the light and dark cycle did not exceed 6 C. However, at a temperature difference of 8 C (HR-4 in Fig. 7), the previously manifested photoperiodicity of emergence changed to thermoperiodicity. In experiment HP (Fig. 5), upon introducing on day 15, a light/dark cycle and exposing the snails to a constant 25 C water temperature, a photoperiodic pattern of emergence became manifested.

These findings indicate that in both cercarial species, light conditions have a greater effect of cercarial emergence than temperature conditions. Furthermore, in *S. haematobium*, photoperiodicity of emergence is more strongly expressed than thermoperiodicity.

Discussion

In the present study we investigated the combined effect of two variables, light and temperature, on the emergence of *S. mansoni* and *S. haematobium* cercariae from their snail hosts during observation periods of at least 14 days. Throughout the experiments, light was a constant intensity of complete darkness (0) or 1,500 lux, and the range of water temperature was from 21 C to 29 C. Therefore, as intensity of natural diffused sunlight gradually rises up to 5,000 lux or more, the possibility that there are other factors like a day-night gradient of light and a certain degree of light, seems to be warranted. And, as snail hosts in tropical areas such as Ethiopia and Kenya are often exposed to the low temperature of 21 C during the night-time, the range of 21–29 C in this study may refer to natural variation in temperature.

It is well known that *S. mansoni* and *S. haematobium* release cercariae during the daytime. Our observations under laboratory conditions showed that cercarial emergence was quite diurnal and photoperiodic in both species (Fig. 1), referring as natural control throughout the experiments in this study.

Cercarial emergence was photoperiodic in both species when their respective hosts were maintained at a constant water temperature of 25 C and exposed to a 12-hr light /12-hr dark cycles (Fig. 2). However, when the experimental conditions were changed after 14 days from a 12-hr light/12-hr dark cycle to continuous illumination, maintaining the same 25 C water temperature throughout, cercarial emergence became nonperiodic in both species (Fig. 3). These findings agree with those of Valle *et al.* (1971, 1973), who reported that the emergence of *S. mansoni* cercariae was nonperiodic when their snail hosts were exposed, at constant temperature, to either continuous illumination or continuous darkness. On the other hand, when their hosts were exposed to continuous illumination and 12-hr cyclic temperature variations of 3 C, the emergence of both *S. mansoni* (MT-1 in Fig. 4) and *S. haematobium* (HT-1 in Fig. 5) cercariae was thermoperiodic. These observations coincide with those reported by Valle *et al.* (1971, 1973), who noted that in *S. mansoni* cercariae, variation in temperature (snail kept in darkness)

can induce a circadian rhythm.

Our results indicate that rhythmic cercarial emergence of *S. mansoni* and *S. haematobium* can be induced individually by either the prevailing light- or the temperature conditions.

Furthermore, we found that cercarial emergence was photoperiodic in *S. mansoni* when their hosts were exposed to a 12-hr light/12-hr dark cycle and reversed cyclic temperature, the difference of which did not exceed 3 C (MR-1 in Fig. 6, MR-4 in Fig. 4). In *S. haematobium*, photoperiodicity persisted to a reversed cyclic temperature difference of 6 C (HR-1, HR-2 and HR-3 in Fig. 7). These findings suggest that while the prevailing light conditions have a greater effect on the diurnal emergence of both species than the temperature, photoperiodicity is more strongly expressed in *S. haematobium* than in *S. mansoni*.

Comparison of the results shown in experiments MN and HN (Fig. 3) revealed that the emergence of *S. haematobium* was markedly reduced during the first 2 days after changing the exposure conditions from 12-hr light/dark cycles at 25 C to continuous illumination at the same water temperature. This suggests that the interjection of a dark period plays an important role in the emergence of *S. haematobium* cercariae. Our recent study (Nojima and Sato, 1982) had revealed that in both species, cercarial emergence is affected by the duration of the preceding dark period.

Pitchford and Visser (1966) and Pitchford *et al.* (1969) referred to diurnal nature in the emergence of *S. mansoni, S. haematobium* and *S. bovis* cercariae; diurnal that in *S. mattheei*, with some nocturnal shedding between September and June (greatest 28% in January); nocturnal that in *S. rodhaini*. As to the emergence of *S. japonicum*, it seemed that there were some strain differences in cercarial nature; they were diurnal in Philippine (leyte) strain, diurnal in Chinese strain and nocturnal in Japanese (Kurume) strain of *S. japonicum*, reported by Nojima *et al.* (1980), Mao *et al.* (1949) and Kifune and Takao (1970), respectively. To date, it is unknown the diurnal or nocturnal emergence of *Schistosoma* cercariae from their snail hosts is circadian. However, our present study suggests that under field conditions, the diurnal emergence of *S. mansoni* and *S. haematobium* cercariae is affected by the prevailing light- and temperature conditions and that the rhythm is not circadian, if circadian rhythm is defined as by Pittendrigh (1960) who stated that circadian nature is innate and/or endogenous and almost completely temperature-independent. This hypothesis regarding the noncircadian nature of cercarial emergence was also supported by our previous study (Nojima and Sato, 1978) and our recent study (Nojima and Sato, 1982). The former indicated that the hourly emergence of *S. mansoni* and *S. haematobium cercariae* after inception of the photoperiod was affected by the prevailing temperature and that the emergence peaked at an earlier hour at the higher temperature, and the latter showed that the hourly emergence of both cercarial species during the photoperiod was affected by the duration of the preceding darkness period and that the hourly emergence was nearest the regular pattern during the photoperiod which had been preceded by a darkness period ranging from 10 to 14 hours.

Summary

The cercarial emergence of *Schistosoma mansoni* from *Biomphalaria glabrata* and *S. haematobium* from *Bulinus globosus* was photoperiodic when the snails were exposed to 12-hr light/dark cycles at a constant water temperature of 25 C. However, when their hosts were subsequently exposed to continuous illumination, cercarial emergence became nonperiodic. Temperature-

induced periodicity was manifested when the hosts were maintained at continuous illumination and exposed to 12-hr cyclic temperature variations of 3 C. Photoperiod inversion resulted in photoperiodic emergence of *S. mansoni* if the cyclic temperature difference did not exceed 3 C and of *S. haematobium* up to a cyclic temperature difference of 6 C. In both cercarial species, rhythmic emergence could be induced by either prevailing light- or temperature conditions, and the illumination affected emergence more than the temperature, especially in *S. haematobium*.

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References

1) Asch, H. L. (1972): Rhythmic emergence of *Schistosoma mansoni* cercariae from *Biomphalaria glabrata*: Control by illumination. Exp. Parasit., 31, 350–355.
2) Giovannola, A. (1936): Inversion in the periodicity of emission of cercariae from their snail hosts by reversal of light and darkness. J. Parasit., 22, 292–295.
3) Glaudel, R. J. and Etges, F. J. (1973): The effect of photoperiod inversion upon *Schistosoma mansoni* cercarial emergence from *Biomphalaria glabrata*. Int. J. Parasit., 3, 619–622.
4) Kifune, T. and Takao, Y. (1970): Factors influencing the release of *Schistosoma japonicum* cercariae from *Oncomelania nosophora*. Jap. J. Trop. Med., 11, 48–49.
5) Luttermoser, G. W. (1955): Studies on the chemotherapy of experimental schistosomiasis. III. Harvest of *Schistosoma mansoni* cercariae by forced nocturnal emergence from *Australorbis glabratus*. J. Parasit., 41, 201–208.
6) Mao, C. P., Li, L. and Wu, C. C. (1949): Studies on the emergence of cercariae of *Schistosoma japonicum* from their Chinese snail host, *Oncomelania hupensis*. Amer. J. Trop. Med., 24, 937–944.
7) Nojima, H., Santos, A. T., Blas, B. L. and Kamiya, H. (1980): The emergence of *Schistosoma japonicum* cercariae from *Oncomelania quadrasi*. J. Parasit., 66, 1010–1013.
8) Nojima, H. and Sato, A. (1978): The emergence of schistosome cercariae from the snails. 1. Hourly response of cercarial emergence of *Schistosoma mansoni* and *S. haematobium*, and effect of light-cut on their emergence. Jap. J. Parasit., 27, 197–213.
9) Nojima, H. and Sato, A. (1982): *Schistosoma mansoni* and *S. haematobium*: Emergence of schistosome cercariae from snails with darkness and illumination. Exp. Parasit., 53, in press.
10) Oliver, L. (1951): The influence of light on the emergence of *Schistosomatium douthitti* cercariae from their snail host. J. Parasit., 37, 201–204.
11) Pitchford, R. J. and Visser, P. S. (1966): The activity of various schistosome cercariae in outdoor aquaria. Ann. Trop. Med. Parasit., 60, 581–521.
12) Pitchford, R. J., Meyling, A. H., Meyling, J. and Du Toit, J. F. (1969): Cercarial shedding patterns of various schistosome species under outdoor conditions in the Transvaal. Ann. Trop. Med. Parasit., 63, 359–371.
13) Pittendrich, C. S. (1960): Circadian rhythms and the circadian organization of living systems. Cold Spring Harbor Symposia Quantitative Biology, 25, 159–186.
14) Valle, C. M., Pellegrino, J. and Alvarenga, N. (1971): Ritomo circadiano de emergencia de cercarias (*Schistosoma mansoni–Biomphalaria glabrata*). Revista Brasileira de Biologia, 31, 53–63.
15) Valle, C. M., Pellegrino, J. and Alvarenga, N. (1973): Rhythmic emergence of *Schistosoma mansoni* cercariae from *Biomphalaria glabrata*: Influence of the temperature. Revista do Instituto de Tropical de Sao Paulo, 15, 195–201.

セルカリア遊出に関する研究
(3) マンソン及びビルハルツ住血吸虫セルカリアの遊出に及ぼす光と温度の相互作用

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Biomphalaria glabrata からのマンソン住血吸虫セルカリアの遊出と，*Bulinus globosus* からのビルハルツ住血吸虫セルカリアの遊出において，両種セルカリアとも，一定温度 (25C)，12時間明/12時間暗のサイクル下では明期に集中して遊出する明周期出現性を示すが，引き続いてこの明暗サイクルを無くして連続照明にすると，周期性が無くなった．

両種セルカリアとも，連続照明，12時間高温/12時間低温の温度サイクル下では高温期により集中して遊出し，3C の温度位相差があれば明白な高温周期出現性を示す．

更に一定温度 (25C)，明暗サイクル下に明周期出現性を示している両種セルカリアの遊出に対して，明期を低温に，暗期を高温になるような逆温度位相差を導入・負荷しても，マンソン住血吸虫では 3C，ビルハルツ住血吸虫では 6C の温度差までは明らかな明周期出現性を認め，両種で明暗と温度に対する反応に差があるようだ．

以上から自然界で昼間遊出性を示す両性セルカリアは光と温度の相互作用を受けたものと解される．