

INFLUENCE OF EXPERIMENTAL ILLUMINATION AND SEASONAL VARIATION ON CROSSBREEDING MATING IN THE SNAIL *BIOMPHALARIA GLABRATA*

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The crossbreeding activities of the Schistosoma mansoni vector snail Biomphalaria glabrata were counted in a laboratory aquarium throughout the year under two regimes of 12h light : 12h dark from 7 A. M. to 10 P. M. Mating increased significantly in Autumn and Winter and just missed a significant inverse correlation with temperature and a direct one with locomotion.

Other similar experiments were carried out to compare mating under various illumination conditions in complete daily cycle measurements. Mating counts decreased under the regimes which submitted snail to a total exposure of 12h light and 12h dark during a daily cycle in the following sequence: 12h light : 12h dark alternating hourly with light gradient, 12h light : 12h dark, 1h light : 1h dark and 12h dark : 12h light. Under two constant illuminations, the mating scored less than under the previous conditions, except under 12h dark : 12h light. Under darkness the mating count was lower than under light conditions.

There was no way to differentiate the night and day rhythms of mating on different days in each regime, except for mating under 12h light : 12h dark alternating with light gradient, constant dark and 12h dark : 12h light conditions.

Mating increased in certain light and temperature conditions, in which the intensities should have an optimum value.

Key words: *Schistosoma mansoni* vector snail – *Biomphalaria glabrata* – illumination – seasonal variation – crossbreeding mating – sexual behavior

Reproduction for the schistosomiasis vector snail *Biomphalaria glabrata* was noted to be exceptional, since it resists very hard environmental conditions due to its defensive behavior (Pieri, 1979). Secondly, its capacity to survive is due to its great sexual fertility. Investigations regarding the reproduction of the snail concerning oviposition and hatching are numerous. But, very little is known of how they behave during sexual activities.

Biomphalaria glabrata breeding is achieved through the eclosion of eggs that are fertilized in two ways, cross and autobreeding. It has been proven that, although a hermaphrodite

species, there is a preponderance of crossmating (Brumpt, 1941; Paraense, 1955). The cross-fertilization occurs through the approximation of two individuals during mating and, in general, the active male snail attaches its sole to the left side of the shell of the active female snail; afterwhich, they may initiate copulation. During the sexual act, the penis is visible, making contact with the female genital opening. However, copulation is not always observed during mating. Schall et al. (1985) reported that the frequency of copulation was only $\geq 3\%$ out of $\geq 10\%$ of mating in adult melanic *B. glabrata*. Copulation rarely happens among a group of three snails and is practically impossible without the described behavior (Rey, 1956). Thus, mating, as a pre-copulatory behavior, seems to be a necessary ritual for crossbreeding.

Research supported by CNPq/PIDE (n^{os} 2222.8060/80 and 40.3811/82).

Received August 8, 1987.

Accepted December 21, 1987.

Reproductive potential per snail varies little, even with accelerated maturity, the snail laying approximately 14,000 eggs when in a group or only 10% less when single, by auto-breeding, during its life span (Ritchie et al., 1966). However, both ways of breeding constitute together a necessary step for snail survival. So, for the snail in a group, mating activities can be observed as an important indication of the reproductive potential.

The present study was carried out in our laboratory for a better understanding of the *B. glabrata*'s mating behavior under the influence of different illumination conditions and/or during a period of one year.

MATERIALS AND METHODS

Mating during a period of one year – Six new snails, all coming from the same pond, with shell diameters of about twenty mm, were used in each seasonal experiment. The snails were put in a 54 L aquarium filled with 14 cm of tap water and located in a room where darkness was about 10^{-2} lux. They were fed on a mixture of guinea pig food (30%), dry alfafa (33%), wheat germ (10%), rich dried milk (7%) and CaCO_3 (20%) every Monday, Wednesday and Friday at 10 A.M. About 1g of food was placed randomly within the aquarium. Before each experiment, the water was replaced and surplus food removed. Variations of water temperature were slow and dependent on chamber inertia. Daily variations of temperature were $\pm 0.5^\circ\text{C}$. Mean seasonal values were between 22 and 25°C .

Two light regimes were employed. In the control regime, the tank was illuminated by vertical light alone, being turned on and off at dawn and dusk; this regime was referred to as 12hL : 12hD abrupt (12hL : 12hDa). During the experimental regime, a horizontal light gradient alternated each hour with a vertical fluorescent light of 10^2 lux intensity for the period of expected day time, while at night the horizontal light gradient was turned on alternately with darkness. A horizontal light gradient was established along the length of the tank, by subdividing it along its length into 11 compartments with 10 smoked glass plates, and illuminating from one end with two 20 W fluorescent "daylight" lamps. This regime was referred to as 12hL : 12hD gradual (12hL : 12hDg). The experimental regime offered an

opportunity to test for an optional illumination for mating behavior. Data were recorded from Spring 1976 to Spring 1977 in the middle of the seasons. More details could be found in Pimentel-Souza et al. (1984), in which a similar experiment was carried out.

The data obtained were plotted according to time. Six records were taken every odd hour coinciding with the horizontal gradient in the experimental regime. Each datum was independently and simultaneously recorded by two observers, totalling seven observers. Records were confirmed by inter-observer's reliability calculated by the "Agreement Index", AI, for each behavioral class (Bijou et al., 1968). Only about 1% of the AI was less than 70% and, as an average, the AI was close to 100%.

Mating under different illumination – The experimental animals, aquarium and alimentation were similar to that described in the previous section concerning mating during the period of one year. Snails were kept in a group before and during the experiment.

The water temperature, which was maintained almost constant ($\pm 0.5^\circ\text{C}$ during daily variation) due to inertia of the experimental chamber, was sampled in a corner of the aquarium at the beginning and end of each observation hour. Variations throughout the first series of experiments were from 22.0 to 25.0°C and, throughout the second series, from 23.0 to 25.0°C .

The experimental illumination conditions were carried out to allow a comparison between four different regimes of a total exposition of 12 hours light and 12 hours darkness in a daily cycle, or between two different constant illuminations and a horizontal gradient of lighting, where snails could find an optional illumination.

The first series of experiments consisted of counts during continuous 48 hour periods, after adaptation for two days before each experiment, during the period from November 17 to December 3, 1978. Four experimental conditions were used:

- 1) 12 hours light : 12 hours darkness abruptly changed, coincident approximately with solar rhythm, referred to as 12hL : 12hDa;
- 2) constant light, referred to as LL;
- 3) constant darkness, referred to as DD;

- 4) 12 hours darkness : 12 hours light, in an inverse solar rhythm, referred to as 12hD : 12hL.

Snails received about 10^2 lux under artificial vertical illumination and 10^{-2} lux under darkness. Twilight was synchronized in solar rhythm by means of a light dependent resistor system, IDIM, at the level of about 10^2 lux of natural illumination.

The second series of experiments consisted of measurements during 72h periods, from January 19 to February 5, 1979, with an adaptation period of two days before each experiment. Three experimental conditions were used:

- 1) 12hL : 12hDa;
- 2) 12 hours light : 12 hours darkness alternated with a horizontal light gradient at odd hours, herein referred to as 12hL : 12hD gradual or 12hL : 12hDg;
- 3) 1 hour light : 1 hour darkness, 1hL : 1hD.

Records were collected in alternate odd hours, when observations were taken every 5 minutes. In these series of experiments, each time only one of the previous year-long experiment trained observers recorded data during the whole one-hour period.

RESULTS

One-year long variation – The one-year long mating variations were examined under 12hL : 12hD gradual, for the experimental and adaptation weeks, and under 12hL : 12hD abrupt, for the control week. Total mating counts in each season were compared between paired seasons and classified in two groups in accordance with the statistical analysis: (a) those with a significant difference between the pairs: Summer X Autumn (heterogeneity test, $\chi^2 = 9.72$, $P < 0.01$); Summer X Winter ($\chi^2 = 4.88$, $P < 0.05$) and Spring X Autumn ($\chi^2 = 5.77$, $P < 0.025$); (b) with a non significant difference between the pairs: Spring X Summer ($\chi^2 = 0.00$); Autumn X Winter ($\chi^2 = 1.51$) and Spring X Winter ($\chi^2 = 2.49$). In conclusion, mating increased significantly in Autumn and Winter in relation to Spring and Summer (Fig. 1).

On the other hand, mating counts under 12hL : 12hD gradual had just missed significance in inverse correlation with the mean temperature along the year (linear regression,

$r = -0.63$, $P = 0.10$, all populations were normal at least at $P < 0.01$ in t-Student test).

In the daily cycle, mating counts per hour in the year-long experiment just missed significance to a direct correlation with locomotion, described in Pimentel-Souza et al. (1984) (linear regression, $r = +0.64$, $P = 0.09$) under 12hL : 12hDg. However this correlation was significant during Winter ($r = +0.73$, $P = 0.05$, the two distributions involved were normal, by the t-Student test).

Under horizontal light gradient, 12hL : 12hDg, mating snails tended to be located in the brighter subdivision of an aquarium in Winter (Fig. 2). This effect is a consequence of photo-positive reaction (Schall et al., 1985) and photokinesis, as shown in Figure 5 in Pimentel-Souza et al. (1984). The hypothesis of random collisions to aggregate *B. glabrata* (Simpson et al., 1963) was confirmed in the increased counts in the lower number subdivisions, which were brighter under 12hL : 12hDg in relation to continuous illumination under 12hL : 12hDa. This fact was specifically true during Winter, when locomotion values were higher. Total mating counts also increased significantly along aquarium subdivision under 12hL : 12hDg, in the experimental week ($\chi^2 = 42.3$, $P \ll 0.001$ in heterogeneity test), and even in the adaptation week ($\chi^2 = 29.8$, $P < 0.001$), in relation to 12hL : 12hDa, during the control week. Under 12hL : 12hDa, the snails mating seemed randomly distributed along the aquarium (Fig. 2). In conclusion, the mating count was dependant on chance variations of collisions, which increased with local population density, depending on the attracting effect of light and other factors such as chemical substances and temperature (Michelson, 1960; Townsend, 1973, 1974; Uhazy et al., 1978).

Illumination influence – Mating during the period of one year showed a significant difference among counts under 12hL : 12hDg (134) and 12hL : 12hDa (78) ($\chi^2 = 15.6$, $P < 0.05$ in heterogeneity test). Stability was found under light gradient conditions, because there was no difference among mating records in adaptation (96 counts) and experimental (134 counts) weeks ($\chi^2 = 6.98$, $P > 0.10$). In conclusion, there was an increasing effect of mating under light gradient condition under 12hL : 12hDg or a depressor effect under lights of 10^2 or 10^{-2} lux under 12hL : 12hDa.

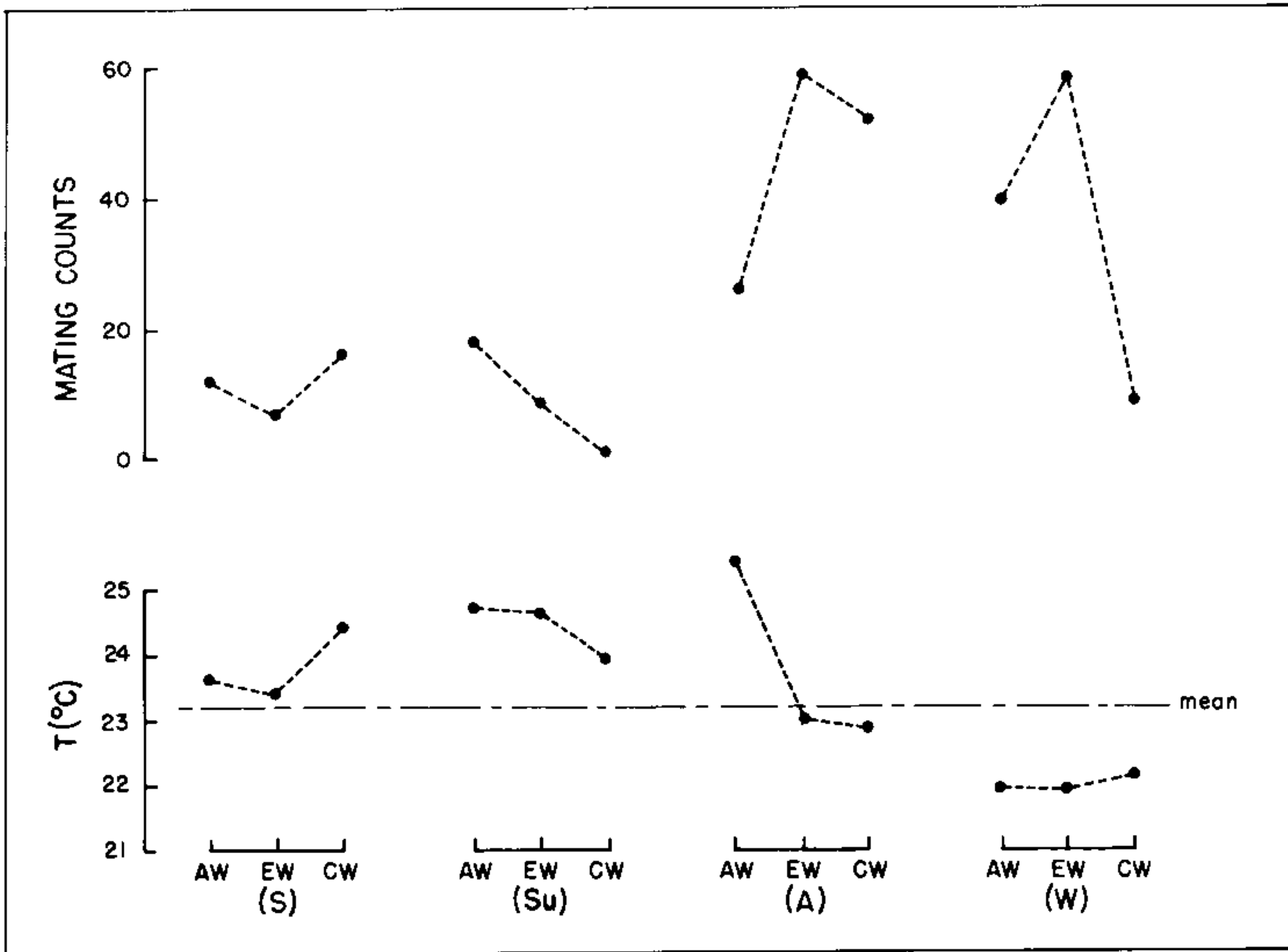


Fig. 1: The variation of mating counts (upper curves) with corresponding mean temperatures (lower curves), measured in three weeks: adaptation (AW), experimental (EW) and control (CW), in each season: Spring (S), Summer (Su), Autumn (A) and Winter (W), during the course of one year in *Biomphalaria glabrata*. Snails were under two illumination conditions: in CW, under 12h light : 12h dark and in AW and EW, under 12h light : 12h dark, alternating with a horizontal light gradient, each hour of observation.

On the other hand, in the first series of experiments under different illuminations, the mating counts of 6 snails amounted to 864 observations. There was no way to differentiate the 1st. and 2nd. days by the day and night rhythm under 12hL : 12hDa ($\chi^2 = 0.00$, in the heterogeneity test) and under LL ($\chi^2 = 1.39$). Thus, under the two previous conditions the day or the night rhythms of activity were stable. However, there was a significant difference between the two stable day and night rhythms of activity under 12hL : 12hDa and LL ($\chi^2 = 14.7$, $P < 0.001$). As in 12hL : 12hDa, periodic activities were similar, 49 and 45 counts for day and night periods, respectively: under LL, night mating counts decreased considerably at night periods (59 to 13). On the other hand, under DD ($\chi^2 = 32.2$, $P < 0.001$) and under 12hD : 12hL conditions ($\chi^2 = 43.1$, $P < 0.001$) the matings rhythms were no longer regular; there was no way to characterize the

day or the night rhythms of mating counts because they were so unstable in each condition. In conclusion, the total counts of mating became reduced, and daily rhythm tended to be unstable in the following sequence of illumination conditions: 12hL : 12hDa (94 counts), LL (72), DD (63) and 12hD : 12hL (48).

In the second series of experiments under different illuminations, the mating counts of 6 snails were constituted of 1,296 observations. The rhythm of activity confirmed that there is not any way to differentiate the 1st., 2nd. and 3rd. days by the day and night rhythms of mating under 12hL : 12hDa ($\chi^2 = 5.47$, $P > 0.05$), but mating count was slightly higher during the night period (115 against 107). The same result was obtained under 1hL : 1hD ($\chi^2 = 3.13$, $P > 0.10$, with 90 and 100 counts in day and night periods respectively). However, night and day rhythms were differentiated

under 12hL : 12hDg ($\chi^2 = 50.8$, $P < 0.001$), with an increase in day period counts (158 against 124). The mating during the day period did not seem to be stabilized, increasing each cycle (30, 45 and 83), as well as decreasing during the night periods (61, 46 and 17). Again, a considerable increase in mating was recorded under optional illumination conditions of light gradient. However, total mating counts in day and night periods were not significantly different one from the other between 12hL : 12hD abrupt and gradual regimes ($\chi^2 = 2.75$) and between 12hL : 12hD a and 1hL : 1hD regimes ($\chi^2 = 0.00$) and just missed significance between 12hL : 12hDg and 1hL : 1hD regimes ($\chi^2 = 3.08$, $P = 0.08$). In conclusion, these matings under natural rhythm of illumination (12hL : 12hDa) were not significantly characterized to be nocturnal or diurnal; they were slightly diurnal in the first series and slightly nocturnal in the second series of experiments. Mating was favored in an unstable way in both periods under light gradient regime (12hL : 12hDg), while under hourly dark-light illumination, mating reduced slightly in both periods of the day.

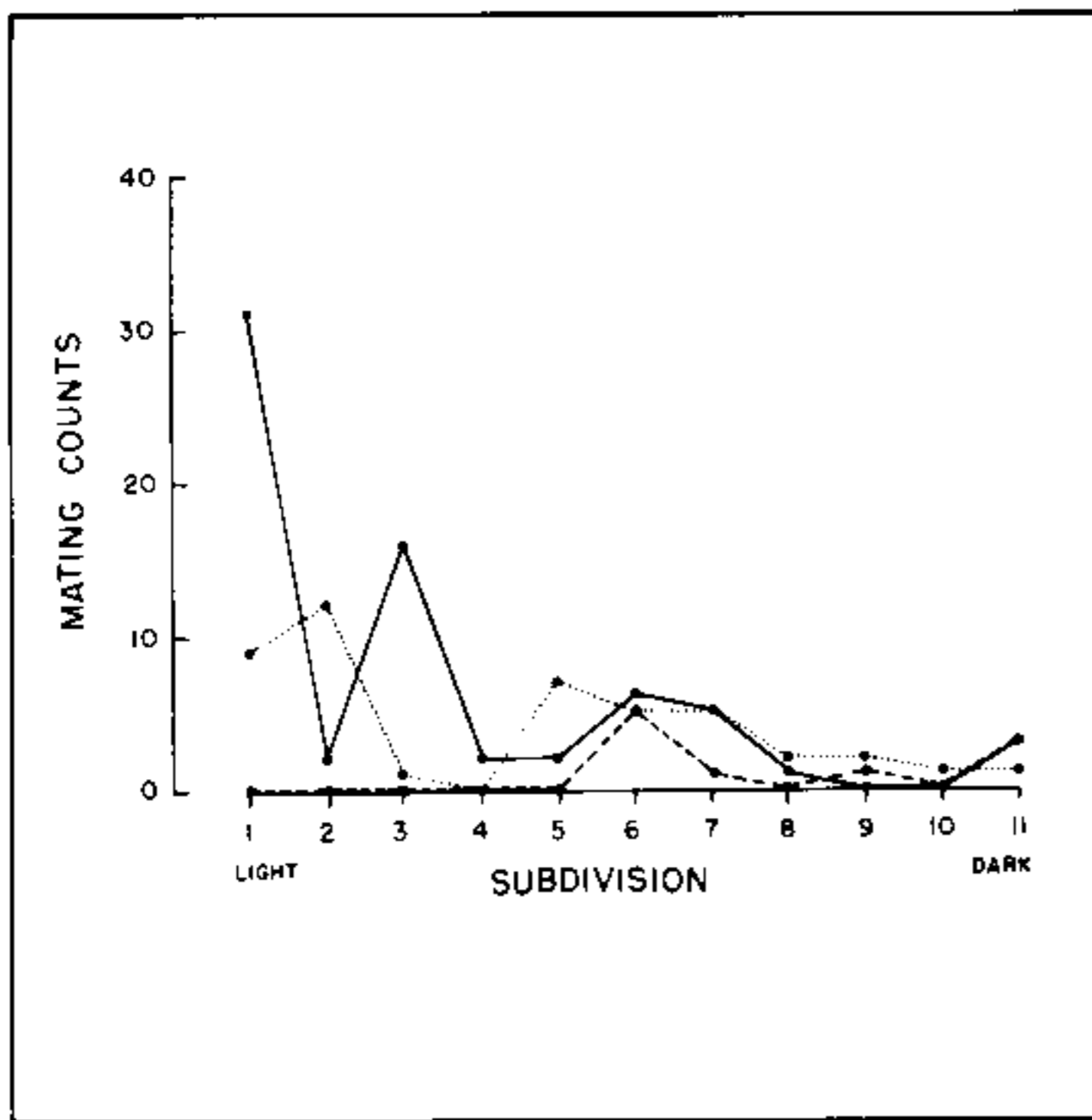


Fig. 2: Mating counts of *Biomphalaria glabrata* within each subdivision of an aquarium, measured during the period of three weeks: adaptation (dotted), experimental (solid) and control (dashed) in Winter. Snails were under two experimental conditions: in control week under 12h light: 12h dark, and in adaptation and experimental weeks under 12h light : 12h dark, alternating with a horizontal light gradient, each hour of observation.

DISCUSSION

Q_{10} for poikilotherms as molluscs are found between 2 and 3, indicating a great effect of temperature on their organic functions and behavior due to the influence in the metabolic rates (Bullard, 1964). In fact, a clear influence of temperature on egg-laying, hatching, growth and survival, particularly for *B. glabrata* and for other schistosomiasis vector snails has already been described (El Hassan, 1974; Freitas et al., 1975; Appleton, 1977; Barbosa, 1984; Barbosa et al., 1987). However, seasonal variation in reproduction rates in *B. glabrata* was dependent on the insertion of the temperature span under study in relation to the preferred temperature (Barbosa et al., 1987 and Pimentel-Souza et al., in preparation). Taking into account Brumpt's (1941) suggestion that temperature is an important daily stimulus to trigger oviposition, in the present work temperature was examined as a seasonal variation trigger. For example, in Autumn, when the temperature was higher, mating was lower, and at the end of the Winter, when the temperature increased slightly, mating reduced. On the other hand, after the decrease of mean temperature in Autumn, mating counts increased and continued elevated until Winter (Fig. 1). The last observation suggests a clear inverse differential effect, in seasons where the mating scores were higher, in spite of a mild absolute influence. In the present experiment, maximum mating counts were obtained between 22 and 23°C, which was the same temperature observed and suggested to be preferred for oviposition (Brumpt, 1941; Barbosa et al., 1987; Pimentel-Souza et al., manuscript in preparation).

Locomotion, taken from Pimentel-Souza et al. (1984), and mating scores seemed alternated in serial intervals of time, in spite of the fact that an hourly variation of mating during the period of one year was not obtained, because hourly distribution was not gaussian on many occasions (t-Student test). Mating counts showed: (1) a minimum from 9 A.M. to 1 P.M. and a peak from 3 P.M. to 7 P.M., just in opposition to the locomotion activities during the course of one year, (2) a peak from 2 A.M. to 7 A.M., which was in opposition to locomotion activities in illumination experiments and (3) a lagging decrease following dusk, corresponding to a lagging increase of locomotion, under the two experimental conditions along one year. This effect confirms the observation that

aggregation in animals (Fraenkel & Gunn, 1961) and mating in snails (Simpson et al., 1973) corresponded to reduced rates of movement and vice-versa. However, this antagonistic relation was not always verified, because both activities also seemed influenced with the inverse of change in temperature. This contradiction appeared to resolve itself when one considers long term samples much greater than the oscillation cycle between the two variables. Mating counts varied positively with locomotion as seen in the tendency of one-year long correlation, but seemed to alternate at short term samples, in daily variation.

During a one week experiment, mating among the individual snails tended to be more frequent between certain pairs in a group of 6 snails. As they changed to constitute other pairs in the following week, individual change of the mating pair tended to balance out the individual mating counts in a close space, even if one has scored low in one week. Confirmation of rarity of copulation in aggregations of three snails (Rey, 1956) is obtained from the fact that just one out of 308 matings occurred in copulation in aggregations of three snails in 10,368 samples during one year.

The sequence of decreasing mating counts per day concerning different illumination conditions in the first series of experiments was: 12hL : 12hDa (47.0); LL (36.0); DD (31.5) and 12hD : 12hL (24.0), and in the second series of experiments: 12hL : 12hDg (94.0); 12hL : 12hDa (74.0) and 1hL : 1hD (63.3). The higher scores of the second series should be justified by the high variability of the reproduction rates in the snail *B. glabrata* (Barbosa et al., 1987). There could be a doubt as to how to classify the performance of the regime 1hL : 1hD in relation to the others in the first series. But, considering that the score of 1hL : 1hD was only 14.5% less than 12hL : 12hDa, while LL was 24.4% less, we should consider mating under 1hL : 1hD counts as showing a better performance than LL.

ACKNOWLEDGEMENTS

We thank GIDE, Federal University of Minas Gerais for supplying snails and CNPq for the scholarships of V. T. Schall and N. D. C. Barbosa. D. L. Nelson for the english revision of the manuscript and N. Fernandes-Souza for the help with the measurements.

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