

ORIENTATION TO LIGHT OF JUVENILE AND ADULT FORMS OF MELANIC AND ALBINO POPULATIONS OF *BIOMPHALARIA GLABRATA* (SAY, 1818)

V.T. SCHALL, P. JURBERG & M.C. VASCONCELLOS

The process of light orientation by the snail Biomphalaria glabrata was studied using the selection technique in a Y-shaped aquarium under vertical or horizontal lighting schemes. Snail behavior was measured on the basis of distance (cm) covered per hour, direction of locomotion, and location of the animal in the aquarium. A comparison was made of the action of the light stimulus on young and adult animals of albino populations from Santa Luzia (State of Minas Gerais, Brazil) and of a melanic population from Touros (State of Rio Grande do Norte) studied in groups and separately. All groups studied were attracted to light. Analysis of the data suggests the existence of two orientation mechanisms with respect to light in these animals, i.e. high photo-orthokinesia and positive phototaxis, which influence their motion in the environment. This evidence permitted us to discuss features of the distribution dynamics of these mollusks in the environment and their relationship with the larval phases of Schistosoma mansoni, for which they act as intermediate hosts.

The effect of light on the activity of *Biomphalaria glabrata* has been the subject of extensive field observations and experimentation (Deschiens & Bijan, 1956; Deschiens, 1957; Harry & Aldrich, 1958; Milward-de-Andrade, 1959; Pimentel & White, 1959; Joy, 1971 a, b; Sodeman, 1973; Sodeman & Dowda, 1974; Pimentel-Souza et al., 1976; Schall, 1980; Pieri & Jurberg, 1981; Rotenberg, Jurberg & Pieri, 1981). Other studies have been carried out to determine the types of artificial light and their respective intensity levels that appear to be attractive to these snails and that may be used in traps for the study of *B. glabrata* ecology (Williams & Coelho, 1973; Pimentel-Souza, 1977). Several studies have also demonstrated the influence of light of the relationship between the larval phases (miracidium and cercaria) of *Schistosoma mansoni* and *B. glabrata*, which appears to affect the parasite entry into and exit from the host mollusk (Lutz, 1918; Kloetzel, 1958; Wright, 1959; Chemin & Dunavam, 1962; Etges & Decker, 1963; Smyth, 1966; Prah & James, 1978). However, the orientation processes used by these mollusks to move in relation to light have not yet been elucidated, although they have been studied in other gastropods (Pelsener, 1935; Warden, Jenkins & Werner, 1940; Milne & Milne, 1956; Carthy, 1969; Fraenkel & Gunn, 1961; Charles, 1966; Kerkut & Walker, 1975). Some species of this class are known to respond to light and have been studied in depth in terms of kinesis, taxis, menotaxis and orientation with respect to the vibration plane of polarized light (Charles, 1966).

For the purposes of the present investigation, the classification of the orientation reactions reported by Fraenkel & Gunn (1961) and Hinde (1970) was used as reference. Since these reactions can vary in the same species because of age (Fraenkel & Gunn, 1961), animals from two different age ranges (young and adult) were studied. In addition, young and adult *B. glabrata* are known to differ in several aspects such as "chemoreception niches" (Thomas & Assefa, 1979) and lamella formation and diapause, since the populations came from seasonal habitats (Paraense, 1957; Pieri & Thomas, in press). Albino specimens were also studied separately because of their lack of pigmentation in visual receptors, which may result in behavioral difference in relation to light stimulation.

In the present study there was investigated the effect of light on the behavior of *B. glabrata* in a situation of selection and attempted to elucidate specifically the following questions: 1) whether light is an attracting factor for both young and adult snails; 2) time taken by the animals to approach the light source; 3) type of orientation process towards lights (kinesis, taxis, or other); 4) whether group factors such as "tendency to aggregate" (Simpson, Thomas & Townsend, 1973; Thomas, 1977) or "mucus trail tracking" (Townsend, 1974; Bousfield et al., 1981) interfere with orientation to light.

MATERIAL AND METHODS

Animals: *B. glabrata* snails were grown from specimens kindly provided by Dr. W. Lobato Paraense (Department of Malacology, Instituto Oswaldo Cruz). Albino samples were from Santa Luzia (State of Minas Gerais), and melanic samples from Touros (State of Rio Grande do Norte). The albino snails were of the type with unpigmented eyes and mantle. Before the experiments, the snails were raised in 5 litre crystallizers from egg capsules laid on the same day. After two weeks, the animals were transported to glass aquaria (70cm x 30cm by 45cm high) containing water that had been previously stabilized for one month. This procedure permitted us to obtain animals of known age. Each aquarium contained a substrate consisting

of 1.5:2:10 calcium carbonate, oyster meal and dirt. The aquaria were maintained in the laboratory under 12 hour light and 12 hour dark conditions. Light was provided by daylight fluorescent lamps placed in pairs about 8cm above each aquarium. Temperature was relatively constant (22 to 25°C) and food consisted of fresh lettuce provided every two days and always available to the animals.

The young specimens were about one month old, with a shell diameter of 5 to 8 mm, and the adults were three months old, with a shell diameter of 11 to 14 mm.

All 80 animals tested as a group were marked with quick-drying epoxy for individual identification by light symbols traced on the upper surface of the last coil in the shell.

Equipment: the experiments were carried out in a room with controlled temperature and luminosity. Temperature was maintained relatively stable ($23 \pm 2^\circ\text{C}$) using two air conditioners connected to a Sernar "Cronomat" timer which alternately turned each one on or off every three hours.

Three Y-shaped aquaria made of dark grey rigid PVC were used. Each consisted of a 50cm long central arm and two side arms measuring 70 x 13 x 8cm high throughout. The "Y" was subdivided into 21 areas of 10 cm for better determination of individual snail location, and the external walls of the arms of each aquarium were numbered to identify those subdivisions. The ends of the arms were closed with transparent acrylic plates that permitted the passage of the horizontal light focus (Fig. 1). The aquaria were maintained on tables painted black to avoid light reflections.

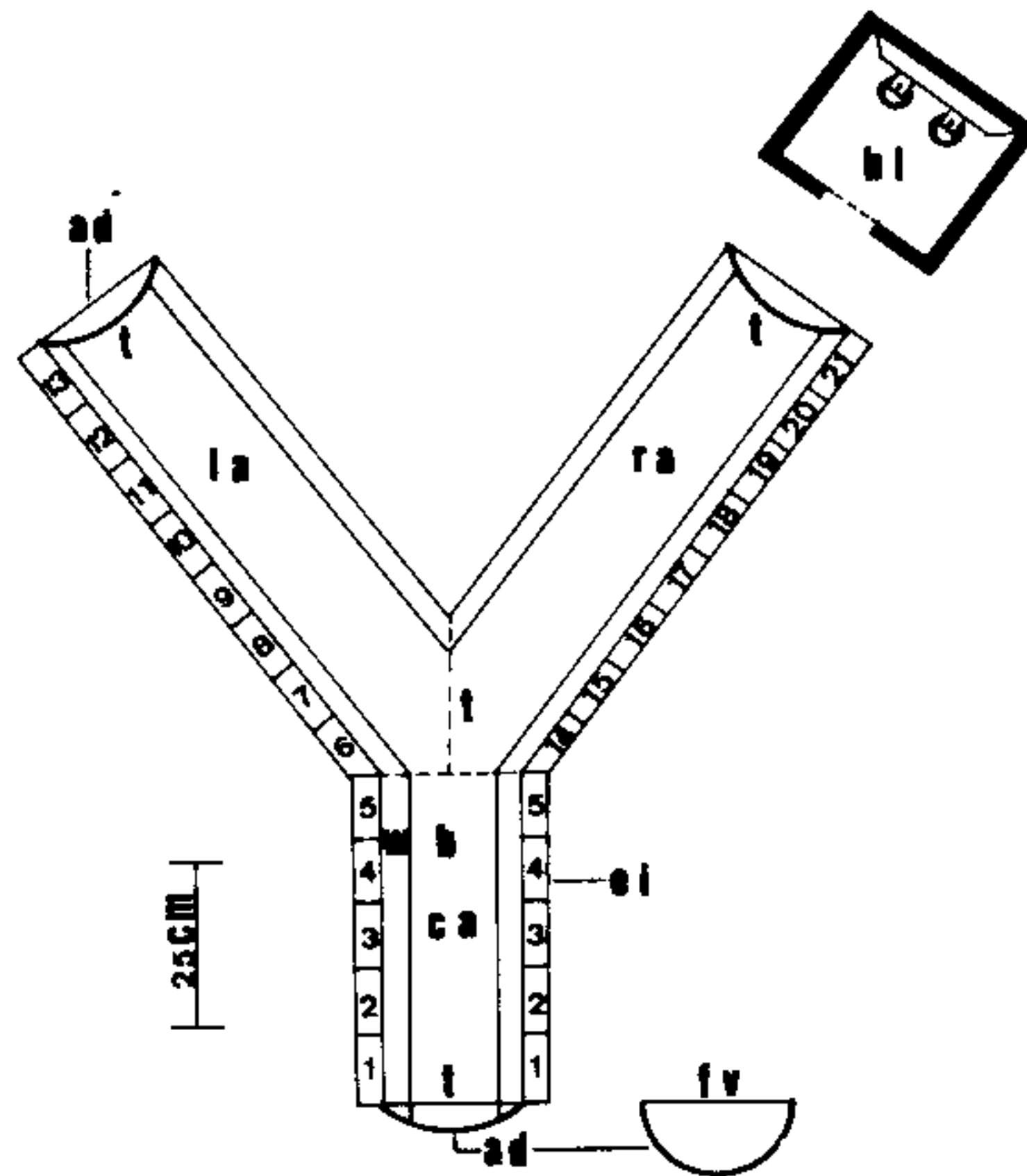


Fig. 1: schematic representation of the Y-shaped aquarium used to study the orientation to light of *B. glabrata*. View from the top. AD: acrylic dish; B: bottom; BL: box with light; CA: central arm; LA: left side arm; RA: right side arm; EI: external identification of areas; FV: frontal view; T: temperature measurement site; W: wall.

On each experimental day the aquaria were filled with unchlorinated water that had been previously stored in asbestos boxes and maintained in the dark for approximately 30 days. The volume of water in each aquarium was 11 litres and 600ml. Water quality was controlled by periodic conductivity (mean values obtained: $100 \pm 18.9 \mu\text{mho/cm}$) and dissolved O_2 ($7.0 \pm 1.0 \text{ mg/l}$) measurements in the reservoirs. After each experiment, the labyrinths were carefully cleaned with neutral detergent (Extran-Merck) and repeatedly rinsed with running water to eliminate any residues left by previously tested animals.

Water temperature was measured at the beginning and the end of each daily test at four different points in the aquaria: central confluence and distalmost ends of the three arms (Fig. 1), with no variations recorded among these points. Water temperature was maintained at $23 \pm 1.5^\circ\text{C}$ throughout the experiments. No variations of more than 0.5°C were recorded from the 1st to the 5th hour.

The experimental light was provided by two daylight fluorescent Philips lamps (color 54) of 20W each, mounted in wooden boxes painted black on the outside and having a circular front opening that could be regulated to vary light intensity (Fig. 1). An opening 5 cm in diameter positioned in front of one of the side arms of the aquarium (sometimes the left, sometimes the right) 10cm from the acrylic plate provided horizontal illumination varying from 0.17 to 350 lux, as estimated by a Gossen photometer, Model Luna-six 3.

In the control situation, illumination was provided by nine 40w bulbs similar to those described above and attached to the ceiling in groups of three at a constant intensity of 350 lux for all three arms of the aquarium.

Procedure: eighty animals were tested in groups of 10 (5 albino and 5 melanic specimens) by the "repeated measurement plan" (Miller, 1977) according to the following experimental design: **Phase I** – two groups of young and two groups of adults were observed for 5 consecutive hours (10:00 a.m. to 3:00 p.m.) over two consecutive days according to the following sequence: 1st day: control situation (CS); 2nd day: experimental situation, with a light focus on the left side of the "Y" (LES); 3rd day: experimental situation, with a light focus on the right side of the "Y" (RES). **Phase II** – two different groups of young and two of adults were submitted to the same procedure as in phase I, but with the sequence of situations inverted (1st day: RES; 2nd day: LES; 3rd day: CS) to avoid possible errors due to order defects (Table I).

TABLE I
Disposition of the tested groups in the Y-shaped aquarium during three consecutive days (Phase I and Phase II) for the study of orientation to light of *B. glabrata*

Age	Young Snails				Adult Snails			
	I		II		I		II	
Phase	I		II		I		II	
Groups	1	2	3	4	5	6	7	8
Melanic	S ₁ – S ₅	S ₁₁ – S ₁₅	S ₂₁ – S ₂₅	S ₃₁ – S ₃₅	S ₄₁ – S ₄₅	S ₅₁ – S ₅₅	S ₆₁ – S ₆₅	S ₇₁ – S ₇₅
Albino	S ₉ – S ₁₀	S ₁₆ – S ₂₀	S ₂₆ – S ₃₀	S ₃₆ – S ₄₀	S ₄₆ – S ₅₀	S ₅₆ – S ₆₀	S ₆₆ – S ₇₀	S ₇₆ – S ₈₀

Obs: S₁, S₂, S₃, S₈₀, represent different snails.

To control possible group variables such as the possibility that the animals would track mucus trails left by themselves or by other individuals (Townsend, 1974), 24 adult animals (3 months of age) were studied separately, each animal being tested in only one situation (CS, LES or RES) in a previously cleaned environment (Table II).

TABLE II
Disposition of the separately tested snails (S) in the Y-shaped aquarium for the study of orientation to light of *B. glabrata*

Situations	Pigmentation	
	Melanic	Albino
CS	S ₁ – S ₆	S ₁₃ – S ₁₈
LES	S ₇ – S ₉	S ₁₉ – S ₂₁
RES	S ₁₀ – S ₁₂	S ₂₂ – S ₂₄

Obs: CS (control situation); LES (left experimental situation); RES (right experimental situation).

No food was provided to the animals during the 5 hours of each test. Each group of 10 individuals or each isolated individual was placed in area no. 1 in the central arm at 10:00 a.m., when behavior recording under the experimental and control conditions was started.

Behavior recording and quantitation: the method used was that of time sampling, whereby pre-selected behavioral categories were recorded from 10:00 to 12:00 a.m. and from 2:00 to 3:00 p.m. Twelve recordings per hours at 5 min intervals were obtained for each animal, using a standardized recording sheet (Table III) that permitted notations for up to 10 individuals per hour referring to the following behavioral parameters: a. Animal location in the numbered areas of the aquarium (1 to 21), the area in which each animal was located being recorded on the sheet every 5 min; b. Location in the internal space of each area, marked as a dot (.) on a square of the recording sheet that represented that particular area. Next to the dot we record the animal's position in one of the four "levels" of the area, i.e., on the wall (W), on the bottom (B), on the surface of the water (S) (subdivided into surface of the water on the wall, SW, floating on the surface, SF, and on the acrylic wall, (AW), and out of the water (O). The reference point used to determine the snail's position was the position of its sole in relations to the environment; c. Contact between animals. Two different conditions of contact were recorded: the sole of one animal on top of the shell of another (OTO), the individuals involved being identified; and occurrence of copulation (C), the individuals involved also being identified; d. Animal locomotion, evaluated as follows: immobility (I); sole sliding on the substrate (S1); floating (F), i.e. with the ventral part of the sole in the water-air interface; or fluctuating (Flu), i.e. with the cephalopodal mass suspended in the middle of the fluid mass.

TABLE III

Model of the standardized recording sheet used to study the orientation to light of *B. glabrata*

Date:	Hour:					Situation:						
Temperature: Initial:	Sample					Light:						
Final:	Observer:					Intensity:						
Minutes	0	5	10	15	20	25	30	35	40	45	50	55
Animals												
1	1 B S1	5B S1	5B S1									
2												
.												
.												
.												

Obs.: (dot that shows the animal's position within one area of the Y-shaped aquarium); B (shows the animal's location on the bottom of the aquarium); S1 (sole sliding on the substrate); 1 or 5 (numbers which identify the area where the animal is in).

These recordings permitted us to measure the following parameters: 1. Time of permanence (tp) in the central arm (CS); total time the animal stayed in areas 1 to 5 before it first reached the side areas, estimated by recording location in the areas every 5 minutes; 2. Distance covered per hour, determined by calculating changes of area as follows: if at minute zero the animal was in area 1 and at minute 5 in area 3, it would have covered approximately 30cm, since each area measured 10cm; 3. Proportion of snails that left the central arm and of snails that moved directly towards the stimulating source without deviations. Direction of locomotion was studied at the central confluence of the Y-shaped aquarium, where an abrupt change in light intensity occurred in the experimental situations. The proportion of exits from the central arm was determined by the sum of the times each individual coming from areas 1 to 5 arrived at the first areas (6 or 14) of the side arms. The proportion of direct locomotion towards the light stimulus was obtained by counting the number of individuals coming from the central arm which turned directly into the initial area of the lighted arm without going through the dark one; 4. All other measurements, such as frequency of the animals in the aquarium arms, location at the different levels in the aquarium (SF, S or O), frequency of contact between animals, were based on the twelve recordings obtained per hour at 5 min intervals, which permitted us to determine the frequency and duration of each item.

During phase I, the data were recorded simultaneously by two independent observers, which permitted evaluation by an "agreement index" (AI) (Bijou, Petersan & Ault, 1968; Pimentel-Souza et al., 1977 and 1984). The index obtained was on average above 97%.

RESULTS

Response to light: the study of animal location in the arms of the Y-shaped aquarium in the control (Fig. 2A) and experimental (Fig. 2B) situation permitted us to compare the effects of light on the behavior of *B. glabrata*. The hypothesis of the attractive effect of light on the samples tested was shown to be correct by statistical analysis of the data concerning individual location in the side segments during the last (5th) hour of recording, when more than 50% of the snails had left the central axis.

In the control situation (CS), which was used as a baseline to determine the spatial-temporal distribution of the animals in the aquarium, random distribution between the left and right side was observed, with no significant difference at the 5% level (Wilcoxon test) for the samples tested as groups, with T = 20 and N = 9 for albino adults (AA), T = 60 and N = 17 for melanic adults (MA), T = 6.5 and N = 8 for albino young (AY) and T = 27.5 and N = 11 for melanic young (MY). A chi-square test applied to the data for albino animals tested separately in the CS also showed no significant difference between the left and right arm ($\chi^2 = 1.65$; d.f. = 1), whereas a significant difference was observed for melanic animals tested separately, with more fixation in the left side arm ($\chi^2 = 8.04$, d.f. = 1, P < 0.05, Fig. 2c).

In the experimental situation (ES), animal location was more frequent in the lighted arm in relation to the dark arm during the 5th hour, with significance at the 5% level (Wilcoxon test) for AA (T = 1; N = 7), MA (T = 3.5; N = 8), AY (T = 2; N = 7), and MY (T = 0; N = 6). Predominance of location in the lighted arm was also significant (chi-square test) for albinos tested separately ($\chi^2 = 53.0$; d.f. = 1), but not for melanic animals tested separately ($\chi^2 = 3.0$; d.f. = 1).

Time for aquarium exploration: by measuring the time of permanence (tp) in the central arm up to the moment when each animal first reached the side arms it was possible to determine when aquarium exploration started for each sample. Table IV shows the tp value for each sample studied in both the CS

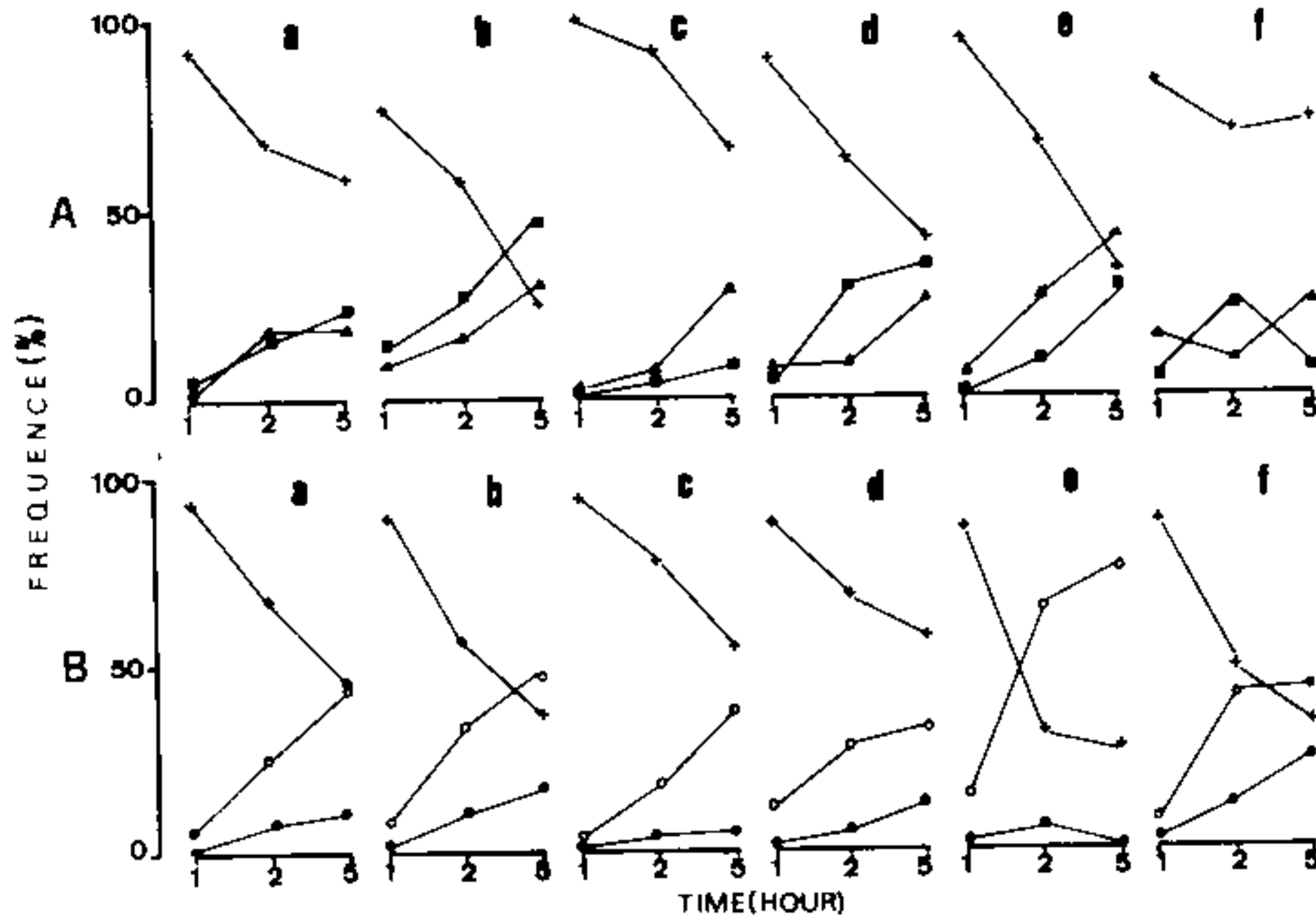


Fig. 2: location frequency (%) of *B. glabrata* in the arms of the Y-shaped aquarium. CA: central arm (+---+); LA: left side arm (■---■); RA: right side arm (▲---▲); IA: illuminated side arm (○---○); DA: dark side arm (●---●), during the three hours of register; A: control situation; B: experimental situation (a: albino adults; b: melanic adults; c: albino young; d: melanic young; e: albino separately tested; f: melanic separately tested).

and ES. When the data for the samples tested as groups (AA, MA, MY and AY) were evaluated by the Kruskal-Wallis test, significant differences in tp were obtained during the CS ($H = 13.84$; d.f. = 3, $P < 0.05$) but not during the ES ($H = 3.42$; d.f. = 3, $P > 0.05$).

When the multiple comparison procedure of Dunn (Hollander & Wolfe, 1973) was applied to the CS, significant differences in tp at the 5% level were obtained only between MA and AY. The tp of the AY group was longer than for all other groups (AA, MY, Table IV) in the CS, although the difference was not statistically significant. The tp of MA was shorter than that of AA and MY, but not significantly so.

TABLE IV

Mean (\bar{X}) and standard deviations (S) of the time of permanence (tp) in the central arm (CA in minutes) in the control situation (CS) and experimental situation (ES) for albino adults (AA); melanic adults (MA); albino young (AY); melanic young (MY); separately tested melanic (SM); separately tested albino (SA)

Situations	CS		ES	
	\bar{X}	S	\bar{X}	S
Animals				
AA	83.0	40.2	70.0	35.2
MA	68.3	46.4	89.0	34.4
AY	114.5	13.9	88.5	38.7
MY	85.0	38.4	84.5	39.9
SA	88.3	38.8	63.3	33.1
SM	66.6	46.8	75.0	41.7

tp values were compared between CS and ES for all samples tested as groups (AA, MA, AY and MY, Wilcoxon-test). The tp of albinos decreased in the ES in relation to the CS, with a statistically significant difference at the 5% level for AY ($T = 0$, $N = 10$), but not for AA ($T = 68.5$; $N = 19$) or AI ($U = 10.5$, n_1 and $n_2 = 6$). The tp of melanic snails increased in the ES in relation to the SC, although not significantly so, for groups MA ($T = 34.5$; $N = 16$) and SM ($U = 17.5$; n_1 and $n_2 = 6$), and did not change for MY ($T = 43.5$; $N = 13$).

Animal dispersal in the Y-shaped aquarium: Side arms – the frequency of animal location in the area 5 of the central arm and in each area of the side arms (6 to 13 on the left side, and 14 to 21 on the right side) during the CS was compared to that for the arms during the 5th recording hour in the ES, when the left and right arms were lighted alternately. Analysis of the data by the Kruskal-Wallis test showed no significant difference in animal dispersal throughout the various areas when the animals were tested as groups (MA, AA, AY and MY) during the CS ($H = 3.49$; d.f. = 3, $P > 0.05$) and the ES ($H = 4.98$; d.f. = 3, $P > 0.05$). In view of this result, the data for all group samples were pooled both for the CS and ES

(Fig. 3A). When the data for animals tested separately were compared by the Mann-Whitney U-test, no significant differences were obtained between SA and SM during the CS ($U = 24.5$; $n_1 = 9$ and $n_2 = 6$) and ES ($U = 23$; n_1 and $n_2 = 8$). Thus, all data for separately tested animals were also pooled (Fig. 3B).

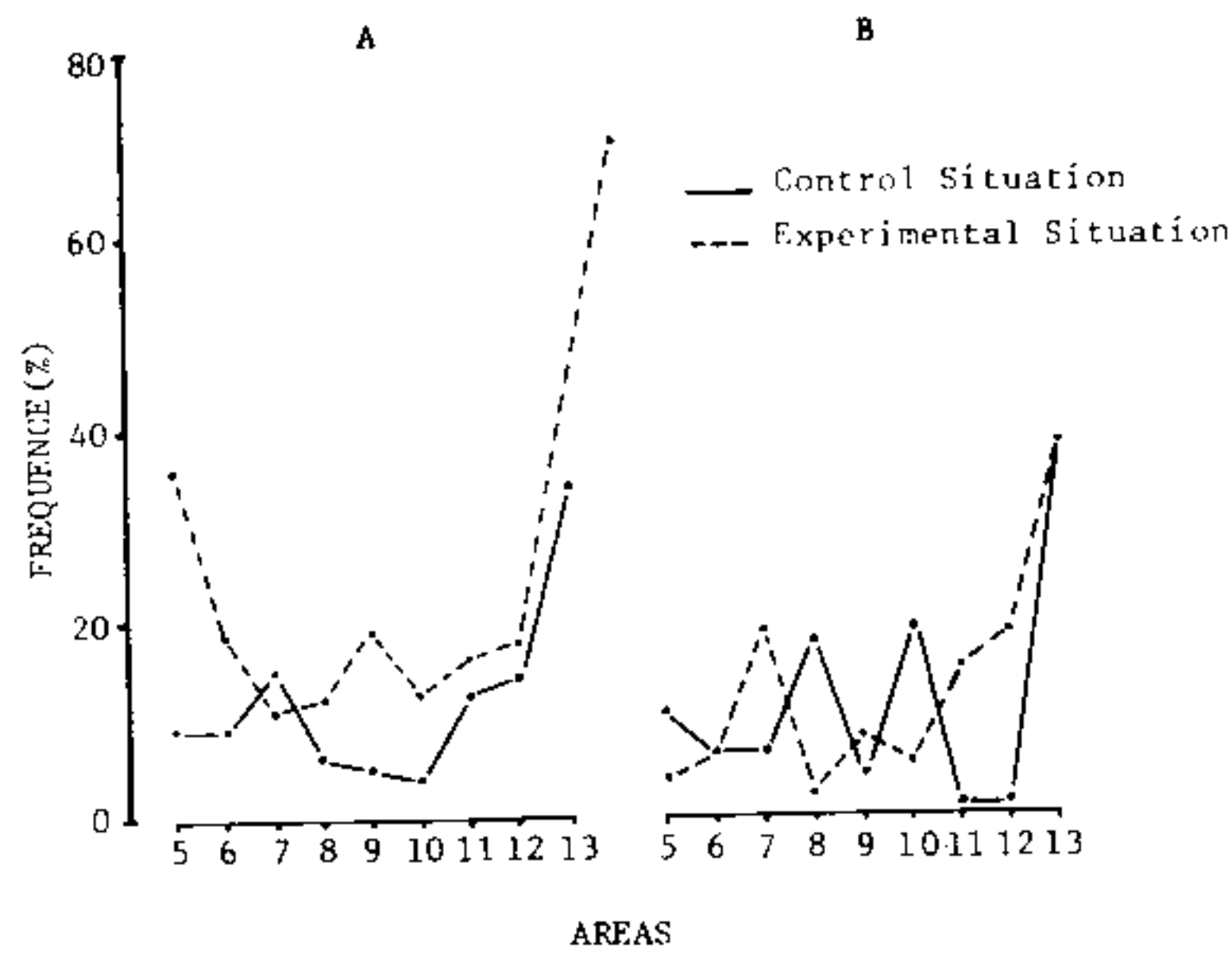


Fig. 3: frequency (%) of *B. glabrata* in the side areas of Y-shaped aquarium in the 5th hour of experiment (2:00 p.m.). A: snails tested in group; B: separately tested snails.

Bimodal distribution was obtained for group samples during the ES, with higher frequency of location in area 5 of the central arm and in the last area of the lighted arm (Fig. 3A). During the ES (separate animals) and CS (both groups and separate animals), higher frequency of location in the last area of the side arms was obtained, indicating a tendency of the animal to fix itself in the most distal end of the arms regardless of stimulation.

When location frequency for separately tested animals was compared between the CS and ES by the Mann-Whitney U-test (Fig. 3A, B), no significant difference was obtained ($U = 42$; n_1 and $n_2 = 9$, $P > 0.05$). When a similar comparison was made for group samples (Wilcoxon test), a significant difference ($T = 3$; $N = 9$; $P < 0.05$) in location frequency in the side arms during the two procedures (CS and ES) was obtained, with a significantly higher location frequency in the last area of the side arm under a horizontal light gradient (70.0%) than during the CS (35.4%), when the side arm was under vertical and homogeneous illumination. **At the various levels in the Y-shaped aquarium (B, W, S (SW and SF) and AW):** to determine animal location at each level in the aquarium (B, W, S (SW and SF) and AW), the data for the twenty animals of each group sample (AA, MA, MY and AY) tested for 3 hours (Fig. 4) were considered as a whole. Individual frequencies for each sample at each level were compared between the CS and ES by the Wilcoxon test to determine the effect of light on vertical animal distribution inside the aquarium. The following results were obtained: 1) higher B frequency in the ES with a significant difference for AA ($T = 8$; $N = 12$; $P < 0.05$) and MY ($T = 10$; $N = 12$; $P < 0.05$); 2) higher W frequency in the CS with a significant difference for AA ($T = 9$; $N = 12$; $P < 0.05$); 3) higher SW frequency in the CS, with a significant difference for MA ($T = 2$; $N = 12$; $P < 0.05$) and AY ($T = 9$; $N = 12$; $P < 0.05$); 4) higher AW frequency in the CS, with a significant difference for MA ($T = 11$; $N = 11$; $P = 0.05$). No significant differences between location frequencies in the CS and ES were obtained for the remaining items and samples.

Animal contact: on the basis of the twelve possible recordings per animal obtained per hour, a small contact frequency (OTO or C) was observed between animals in the group samples (AA, MA, MY and AY). The percent of OTO for adult animals (AA and MA) was $\geq 10\%$, and always $> 10\%$ for young animals (MY and AY, Fig. 6). The frequency of copulation was $\geq 3\%$ for adults only.

To evaluate the effect of light on OTO occurrence, OTO frequency in the CS and ES was compared by the Wilcoxon test, with a significant difference being obtained only for the AA group ($T = 10.5$; $N = 11$; $P = 0.05$), which showed higher OTO frequency in the CS. No significant variations in OTO were obtained for the remaining samples (MA, AY and MY).

Locomotion – variation in time and direction: locomotion was evaluated on the basis of the distance (cm) covered per hour by the animals in the Y-shaped aquarium. A variation as a function of time of permanence in the aquarium was obtained for AA and MA in the group samples and for separately tested animals (SA and SM), which was characterized by a drop in locomotion at 2:00 p.m. after an increase at 11:00 a.m., except for AA. When locomotion data for the three recording times (10:00 a.m., 11:00 a.m. and 2:00 p.m.) were compared for all groups studied by the Friedman test, a significant difference at the 5% level was obtained only for AA in the CS ($\chi^2 = 6.0$; d.f. = 2) and AM in the ES ($\chi^2 = 9.3$).

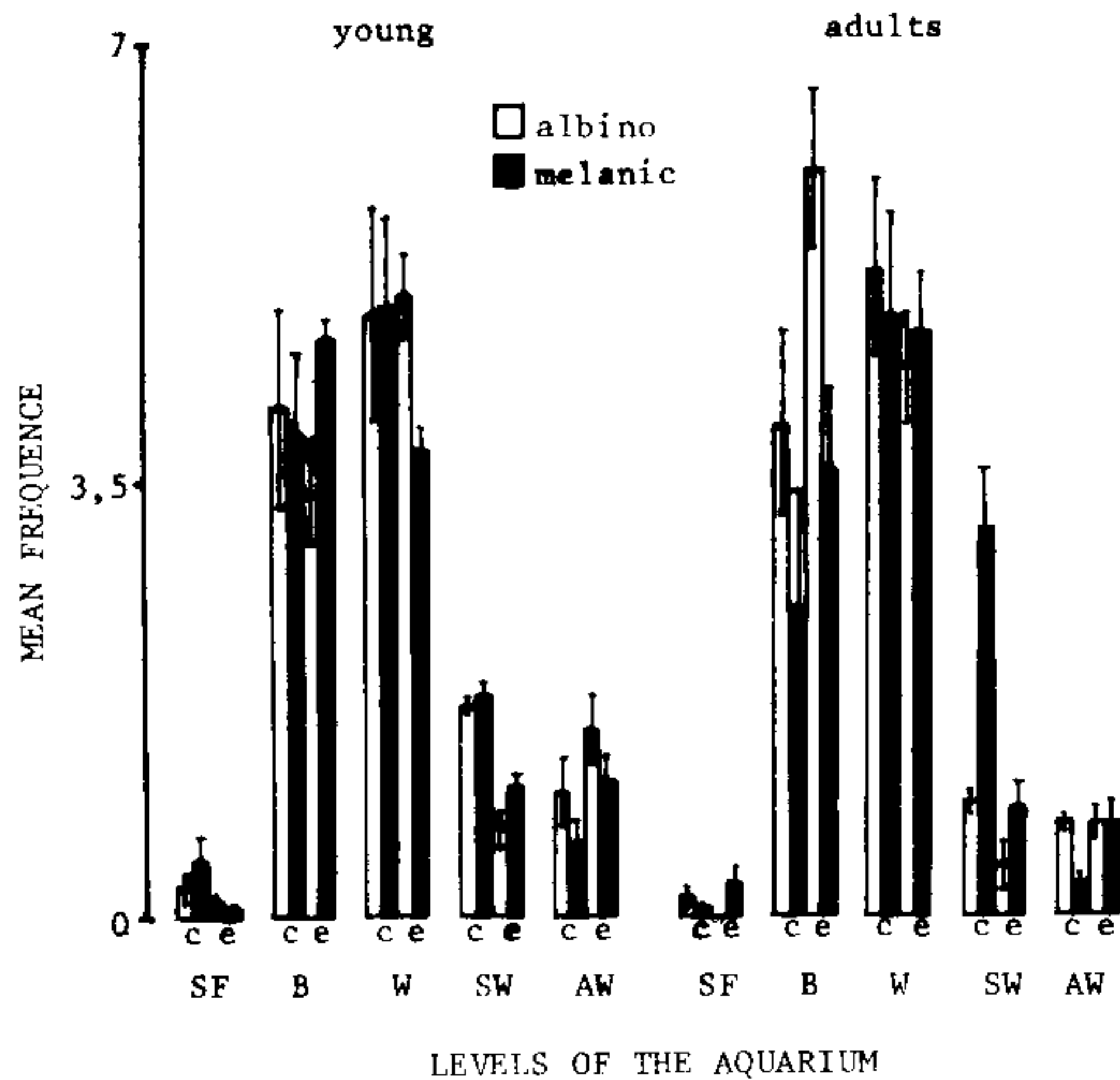


Fig. 4: mean location of 20 snails in three hours of experiment (10:00 a.m., 11:00 a.m. and 2:00 p.m.) in the levels of the Y-shaped aquarium. SF: surface floating; B: bottom; W: wall; SW: surface of the water on the wall; C: control situation; E: experimental situation; AW on the acrylic wall. Lines show the standard deviations.

When locomotion was compared in relation to the experimental condition, higher values were observed for the CS than the ES for melanic samples (Fig. 5), with a significant difference at the 5% level (Wilcoxon test) only for the MA (T = 28, N = 19) and MY (T = 25.5, N = 18) groups at 10:00 a.m.

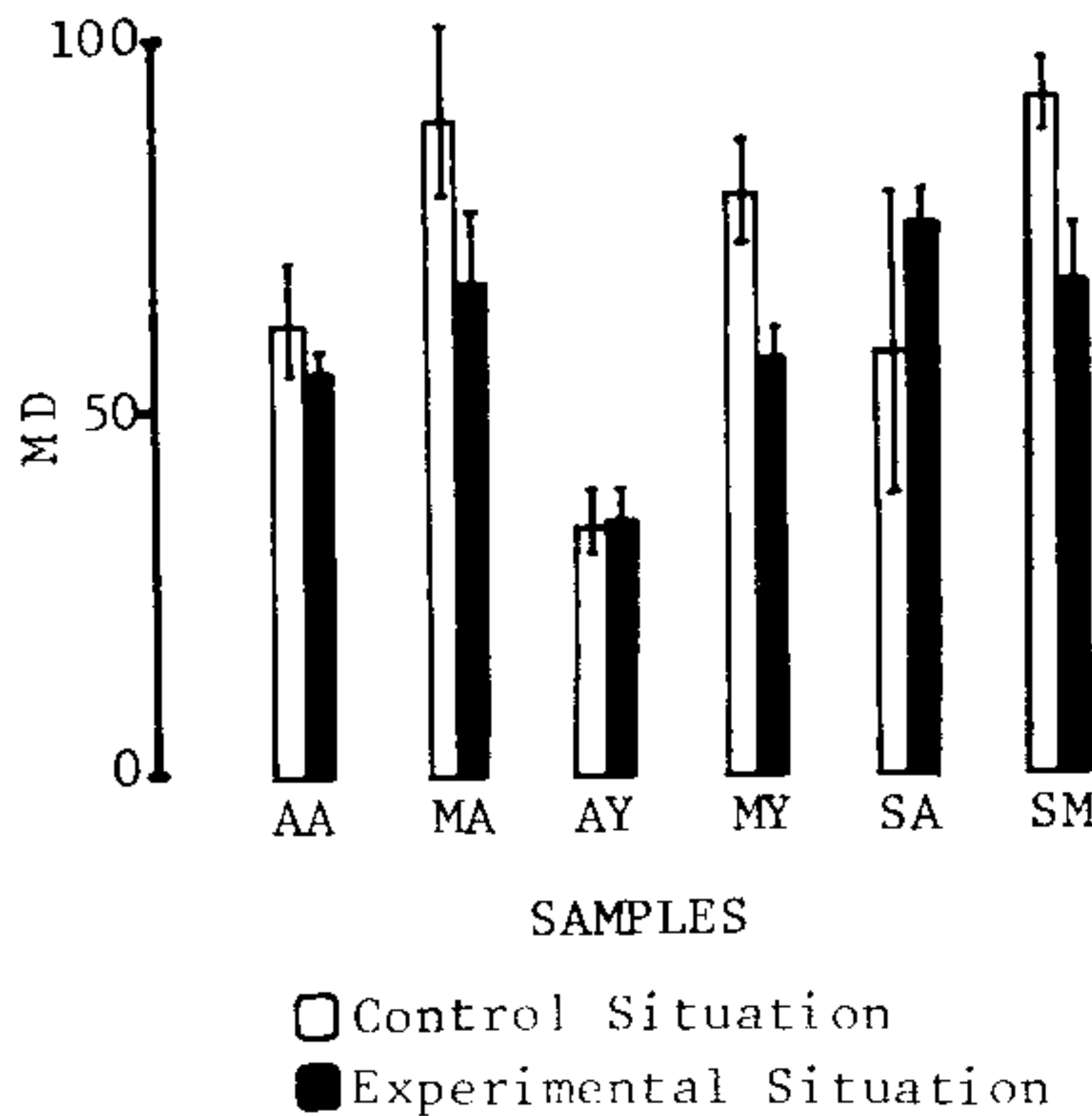


Fig. 5: locomotion (mean distance covered per hour-MD-in cm) in the control situation and experimental situation. AA: albino adult; MA: melanic young; SA: separately tested albino; SM: separately tested melanic.

The locomotion rate of the four group samples in the CS and ES was compared by the Kruskal-Wallis test, with significant differences at the 5% level obtained for the CS (H = 17.53; d.f. = 3) and the ES (H = 20.21; d.f. = 3). The multiple comparison Dunn test showed a significant difference in locomotion between MA and AY and AY and MY (P < 0.05) in the CS, with no significant differences between the remaining groups. In the ES, significant differences were obtained between AA and AY and between MA and AY (P < 0.05). In all cases, the AY sample showed significantly lower locomotion values. When the

data for adult animals (AA and MA) were considered as a whole, a 27% higher locomotion rate was obtained than for young animals (MY and AY). Similarly, melanic animals (MA and MY) had a 35% higher locomotion rate than albino animals (AA and AY).

The direction of locomotion was evaluated to classify the type of motion exhibited by the animals in relation to the stimulus, and consequently, the type of orientation. In the ES, more than 50% of the animals in each of the samples studied (Table V) moved out of the central arm, with a mean of 63.9%. This means that by the 5th hour of exposure to the light stimulus in the ES more than half the animals were able to reach the side arms and choose between dark and light. Among these animals, 81.5%, on average, moved directly toward the light. The chi-square test showed no significant difference at the 5% level in percentage of moving out of the central arm ($\chi^2 = 0.0017$; d.f. = 3) or direct locomotion towards the stimulus ($\chi^2 = 0.547$; d.f. = 3) between the animals in the AA, MA, MY and AY groups. This indicates similar ability of all these groups to orient towards the stimulus by directional movements, which characterize taxis. When adults tested as groups (AA and MA) were compared to separately tested adults (SA and SM) by the chi-square test with respect to moving out of the central arm, no significant differences at the 5% level were found for AA x SA ($\chi^2 = 1.10$) or MA x SM ($\chi^2 = 0.007$). In contrast, significant differences in percentage of direct locomotion towards light were obtained both for AA x SA ($\chi^2 = 4.91$) and for MA x SM ($\chi^2 = 12.93$), with SA showing a higher percentage than AA, and MA a higher percentage than SM (Table V). In both cases, social condition (group or separate testing) interfered with the response to the stimulus although in the opposite way for each sample, with the attraction of albinos decreasing significantly, and the attraction of melanic animals increasing significantly in the group samples.

TABLE V

Number (N) and percentage (%) of snails that left the central arm (CA) and of snails that moved directly towards the illuminated arm (IA) in the experimental situation.

	Samples						Total
	AA	MA	AY	MY	SA	SM	
a) N Total	40	40	40	40	6	6	172
b) N left the CA	28	27	24	22	5	4	110
c) N moved to IA	20	25	20	18	5	2	90
d) % b/a	70.0	67.5	60.0	55.0	83.3	66.7	63.9
e) % c/b	71.4	92.5	83.3	81.8	100.0	50.0	81.8

Obs.: % b/a = percentage of snails that left the CA as related to total of snails tested; % c/b = percentage of snails that moved directly towards the IA as related to number of snails that left the CA.

DISCUSSION

The present results confirmed the positive attraction to light of *B. glabrata* already shown by Willians & Coelho (1973), Pimentel-Souza (1977) and Schall (1980), and also permit us to propose the existence of two different mechanisms of orientation in relation to light in this species, i.e. high photorthokinesia and positive phototaxis. Among the results that support the existence of positive phototaxis are: the significant option by the animals for the lighted arm in the ES, which indicates ability to detect the stimulus and to move toward it; the high rate of locomotion by directional, undeviating movements towards light in the confluence of the Y-shaped aquarium, where transmission of illumination occurred in the ES, a fact that is characteristic of taxis according to the classification of Fraenkel & Gunn (1961). The higher frequency of animals on the bottom associated with horizontal illumination in the ES, and on the wall and surface associated with vertical illumination in the CS may indicate a tendency towards positioning the body in relation to the direction of the light source. This evidence was also observed by Pimentel-Souza et al. (1976, 1984) and by Schall (1980). This attraction, however, cannot as yet be classified into subcategories such as tropotaxis or telotaxis. Later tests using procedures such as the "two-light experiment" and/or the unilateral blindness technique (Fraenkel & Gunn, 1961) will be needed for further classification.

Other features permit us to assume the presence of another mechanism of orientation to light in this species. According to Fraenkel & Gunn (1961) and Hinde (1970), the variation in locomotion rate associated with the variation in stimulus intensity is a specific characteristic of orthokinesia, a mechanism that permits animals to aggregate at sites favorable to their survival. In the present study, a higher locomotion rate was obtained in the CS than in the ES, i.e. under greater light intensity for melanic snails. A similar result was obtained by Sodeman & Dowda (1974) in a study on albino *B. glabrata*. These authors concluded that the concentration of the animals in the dark area (3 lux) of a half-lit arena (1400 lux) did not imply a direct choice of darkness, but may have been the result of an indirect locomotion effect, which may be interpreted as a photokinetic reaction. Sodeman (1973) also obtained a transitory decrease in the locomotion rate of *B. glabrata* by turning off ambient light. The higher locomotion rate associated with greater light intensity may be interpreted on the basis of the presence of the orientation mechanism denoted high photorthokinesia. This type of orientation permits the animal to move away from areas of aversive stimulation

and to stay in areas of adaptive stimulation. We may assume that the light intensity used by Sodeman & Dowda (1974), of the order of 1400 lux, was aversive for the albino specimens used in their study. In contrast, Chernin (1967), using 600 lux illumination, observed that the snails avoided shady areas. Field observation data are apparently contradictory, with some authors reporting higher predominance of *B. glabrata* in shady areas, and others reporting greater aggregation in sunlit areas (Milward-de-Andrade, 1959; Barbosa, 1970). The luminosity values for the areas described as shady or illuminated in these studies are not always reported, with consequent difficulty in data interpretation. In addition, it should be remembered that the circadian seasonal variations in the rhythm of illumination and light intensity cause variations in the intensity values of what is defined as light and shade.

High photo-orthokinesia permits the animal to avoid areas with incidence of high light intensity, which may be aversive not only for their optical component, but also for the high temperatures with which they are generally associated. Data reported by Chernin (1967) have shown that *B. glabrata* snails tend to aggregate in the 27 to 32°C temperature range, avoiding temperatures above and below these levels.

The positive phototaxis observed may be related with vertical motion towards the surface of the water when the animal lives in habitats of a certain depth. Previously reported data permit us to assume that the changes in illumination at twilight may act as a stimulus affecting the activity of *B. glabrata*. Pimentel & White (1959) observed a higher frequency of *B. glabrata* snails on the water surface also obtained by Pieri & Jurberg (1981). This vertical migration may be marked by the change in illumination at twilight. In a study on the circadian rhythm of motion in relation to seasonal variations in illumination, higher indices were obtained around one to two hours after sunrise and sunset, indicating that the transition in environmental illumination affects the activity of *B. glabrata* (Schall, 1980; Pimentel-Souza et al., 1984). Changes in activity associated with changes in illumination have also been observed by Rotenberg, Jurberg & Pieri (1981) in a study of different lighting schemes for *B. glabrata*. It should also be remembered that sunlight only penetrates water at a marked angle, and that during penetration some wavelengths are absorbed and intensity decreases (Wald, 1959). After sunrise and before sunset, the inclination of the light rays is such that they practically do not penetrate water but are reflected by its surface. Thus, light is available only at the surface. It has been observed that certain mollusks at these times move up from the bottom towards light (Buddenbrock, 1970). The same may happen with *B. glabrata*, which, being pulmonate, generally moves towards the surface for gas exchange. In this case, phototaxis would have a high adaptive value because it would leave the animal to the environment that is appropriate for its physiological needs. This is even more relevant during the night period, when the oxygen level in the liquid mass decreases. Thus the decrease in light intensity during sunset may cause the animal to orient towards the surface following the vertical gradient of light intensity. These data lead us to suggest that luminous traps placed on the water surface at night may facilitate a sampling study of these snails in the field in environments such as water reservoirs and ponds, where waters are deeper. Willians & Coelho (1973) demonstrated that luminous traps placed on the surface of an aquarium are more effective than when placed on the bottom or in the middle. In addition, we may assume that the light source should not necessarily have to be inside the trap, a fact that would create manufacturing difficulties and high costs. Illumination could be provided by a focus placed on a support above the water level, under which the trap would be placed on the water surface (Fig. 8).

As to animal age, this factor was differential for albino animals, but not for melanic ones. Young albinos showed lower locomotion rates and longer time of permanence in the central arm in relation to the adult groups (both albino and melanic animals) and even to the young melanic animals. On the other hand, young melanic animals did not differ significantly from the adult groups in any of the behavioral features studied.

When data for melanic and albino animals are compared, it should be remembered that these animals have different origins, leaving open to question up to what point behavioral differences are due to pigmentation or to differences in strains. In any case, albino animals spent less time in the central arm during the ES, significantly so for AY, indicating a differential trophic effect of light in the ES. A higher rate of direct locomotion towards light was obtained also for albinos tested separately in the ES, and therefore without social facilitating mechanisms. In contrast, melanic animals, when tested separately, showed no significant responses to light. These differences suggest that the intensities tested were more attractive for albinos. The fact that these animals have no pigmentation on the retinal layer, which usually prevents light reflection into the inner chamber of the eye, may account for the increased perceptive threshold for low intensities. To confirm this hypothesis, however, it will be necessary to test pigmented and melanic animals of the same strain, as well as homozygous and heterozygous albino specimens (Richards, 1970).

When the observed *B. glabrata* positive phototaxis is compared with the *Schistosoma mansoni* larval phases (miracidia and cercariae) response to light, some convergence is noted. Prah & James (1978) have demonstrated a positive phototaxis and negative geotaxis in *S. mansoni* miracidia. This finding reinforces the idea that miracidia and the molluscan host exhibit similar behavior patterns which bring them into the same layers of environment (Smyth, 1966). Just as Wright (1959) has pointed out, it should be taken into account that the parasites are probably attracted at first, not to a particular host, but to a certain type of environment. This principle may govern the association between *S. mansoni* miracidia and

the snail *B. glabrata*. In this regard, Prah & James (1978) discuss the negative phototaxis and positive geotaxis of *S. haematobium* miracidia that usually spread in the water bottom, where the snail host *Bulinus globosus* is often found.

The *S. mansoni* cercariae show a rhythmic emergence from *B. glabrata*, which is controlled by temperature and illumination, as Asch (1972) and Valle, Pellegrino & Alvarenga (1973) have demonstrated. Several authors have already shown the increase in the number of cercarial release during the brightest hours of the day (Valle, Pellegrino & Alvarenga, 1973). Despite this convergence phenomenon, it is important to know that only a small proportion of the snail population is infected and the cercarial density in the water is low (Warren, 1982). Therefore, more studies are necessary on the rhythmic patterns of these organisms, besides the attraction mechanisms, for a better understanding of the factors involved in the transmission of schistosomiasis.

RESUMO

Com finalidade de conhecer melhor o processo de orientação pela luz do caramujo *Biomphalaria glabrata* foi utilizada a técnica de situação de escolha num labirinto em Y sob esquemas de iluminação vertical ou horizontal. Foram tomadas como medidas de comportamento a distância percorrida (cm) por hora, a direção da locomoção e a localização do animal no labirinto. Procurou-se comparar a ação do estímulo sobre animais jovens e adultos provenientes de populações alvínea de Santa Luzia (MG) e melânica de Touros (RN), estudados em grupo e isolados. Os resultados mostraram um efeito atrativo da luz sobre todos os grupos estudados. A análise dos dados sugere a existência de dois mecanismos de orientação em relação à luz neste animal como alta foto-ortotaxia e fototaxia positiva, os quais devem influenciar seus deslocamentos no meio. Tais evidências permitem discutir aspectos da dinâmica da distribuição desse molusco no ambiente e de seu relacionamento com as fases larvais do *Schistosoma mansoni*, do qual é hospedeiro intermediário.

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