ORIENTATION OF THE SNAIL BIOMPHALARIA STRAMINEA (DUNKER, 1848) IN RESPONSE TO LIGHT IN A SITUATION OF SELECTION

VIRGINIA T. SCHALL, PEDRO JURBERG & BRANI ROZEMBERG

The behavioral response of Biomphalaria straminea to light was evaluated in terms of location of the snail in a Y-shaped aquarium in a situation of selection and of the rate (cm/hour) and direction of locomotion under homogeneous (vertical) or differential (horizontal) lighting upon only one arm of the aquarium. The light source consisted of daylight fluorescent lamps with a spectrum close to that of natural light, with illumination varying from 2.8 to 350 lux. Analysis of the data showed that all animals, whether in groups or isolated, were attracted to light, although the time needed to approach the light source was 50% shorter for the former than for the latter. The rate of locomotion of B. straminea was 35% higher than that observed in B. glabrata and 51% higher than that observed in B. tenagophila studied under similar conditions. The results are discussed in terms of social factors and geographical distribution of the three species.

Key words: Biomphalaria straminea - behavior - light

The objective of the present study was to contribute to the knowledge of the species *Biomphalaria straminea*, an important host snail for schistosomiasis in Northeastern Brazil (Pinotti et al., 1960; Barbosa, 1968; Paraense, 1972), whose behavioral responses have been little studied, with limited bibliography being available as concerns this mollusc.

Studies of this type have been carried out on *B. glabrata* (Chernin, 1967; Townsend, 1974; Pimentel-Souza et al., 1976a, b, 1984; Pieri & Jurberg, 1982; Schall, Jurberg & Vasconcellos, 1985, among others) and on *B. tenagophila* (Schall, Jurberg & Vasconcellos, 1986), providing more in-depth understanding of the host snails by revealing their reactions to stimuli such as light, temperature, pressure, various chemical substances, and social stimulation.

The objective of the investigation of the action of light on the behavior of the species *B. straminea* was to determine the animal's orientation reactions, the possibility of the stimulus functioning as an attractant for the species, the time needed for a response, and the group effects interfering with the type of response shown by the animal.

The study was based on the ethological approach proposed by Hinde (1970) and Hutt & Hutt (1974), and on the categorization of orientation reactions suggested by Fraenkel & Gunn (1961).

MATERIAL AND METHODS

Animals and equipment: we utilized 84 adult melanic noninfected specimens (7 to 10mm in diameter, $\bar{x} = 8.6$, s = 0.77) descending from a sample originating from Picos (PI) and supplied by the Department of Malacology, Instituto Oswaldo Cruz.

Some of the methods used were those standardized in a similar study on *B. glabrata* by Schall, Jurberg & Vasconcellos (1985) i.e. rearing and maintenance of the animals in the laboratory, marking technique for recognizing each individual when the animals were tested in groups, equipment (three Y-shaped aquaria), lighting scheme, control of possible interfering variables, and definition and recording of behavior.

Water temperature was recorded at the beginning and at the end of each daily test at the end of each arm and in the center of the aquarium. For the six experiments in which the animals were tested in groups, mean initial temperature was $22.5 \pm 0.7^{\circ}$ C and mean final temperature was $21.5 \pm 1.2^{\circ}$ C. When the animals were tested separately, initial temperature was $21.8 \pm 0.4^{\circ}$ C and final temperature, $21.6 \pm 0.6^{\circ}$ C. Variation from the first to the last hour of the experiment did not exceed 1° C.

Light intensity was 350 lux in the control situation, and varied from 0.17 to 350 lux in the experimental situation, with values of 2.8 to 350 lux in the lighted arm.

Research supported by CNPq.

Instituto Oswaldo Cruz, Departamento de Biologia, Caixa Postal 926, 20001 Rio de Janeiro, RJ, Brazil. Received for publication November 20th, 1985 and accepted June 9th, 1986.

Procedure: a total of 84 animals were tested, 60 of them in six groups of 10 animals each, and 24 separately. The animals tested as a group were submitted to the "repeated measurement plan" (Miller, 1977) as follows: two groups of 10 animals each were studied in May 1982. On the first day, the animals were exposed to the control situation (CS), i.e. a group in each aquarium. On the following day, one of the groups was submitted to the left experimental situation (LES), i.e. the room was darkened and the light beam fell on the left arm of the Y-shaped aquarium. On the third day, the other group was submitted to the right experimental situation (RES), with light falling on the right arm of the aquarium.

During the month of June 1982, two other groups were tested as follows: on the first day one of them was submitted to RES, on the second day the other was submitted to LES, and on the third day both groups were submitted to CS, one group per aquarium (opposite order to the previous one).

Two other groups were tested in September 1982 following the same scheme as used during the tests of May 1982.

To eliminate possible intertering variables resulting from a group effect, 24 additional animals were studied under isolated conditions between July and August 1982. Three animals were studied per working day, each one isolated in a Y-shaped aquarium similar to those described for the group tests. The following scheme was used:

three animals in CS during the first and fourth day;

two animals in LES and one in RES on the second day;

two animals in RES and one in LES on the third day.

This scheme was repeated for 12 other animals, with the animals similarly submitted to only one of the conditions (LES, RES or CS), i.e. on the basis of independent samples.

The reason why the experiments were performed under LES and RES conditions on the same day was to avoid that a possible fixation of the animals in one arm of the aquarium due to any factor other than light may interfere with interpretation of the responses by favouring the left or right side.

Before the above tests were started, preliminary tests were performed in which the data were collected simultaneously and independently by two observers and an average "agreement index" of 85.8% was obtained between recordings (Bijou, Peterson & Ault, 1968; Pimentel-Souza, 1977).

The following procedure was used in each experimental or control session: the experiment was started at 9:00 a.m., with a group of 10 animals or each animal separately being placed in area number one of the central arm of the Y-shaped aquarium. Starting from that moment, the behavior of the animals was recorded every five minutes during the first (9 to 10), third (11 to 12), fifth (12 to 13) and seventh (15 to 16) hour. After seven hours of permanence in the aquarium, the snails were removed and the aquarium was washed in order to eliminate mucus and residues left by the animals that might interfere with the subsequent sessions.

No food was offered to the animals during each session.

The following items were investigated in the study:

- 1 -location of the animal in the areas of the aquarium
- 2 distance covered per hour (cm)
- 3 direction of locomotion with respect to light
- 4 time of permanence in the central arm of the aquarium.

The method used for recording and quantifying these parameters has been described by Schall, Jurberg & Vasconcellos (1985).

RESULTS

Analysis of the behavioral items studied by the coefficient of variation showed the existence of two subgroups within the six groups of 10 animals tested in the control and experimental situations. One of them, consisting of four CS and four experimental situations (ES), representing 68% of the animals testes in groups, was submitted to joint analysis owing to its similar behavioral characteristics (Sample 1).

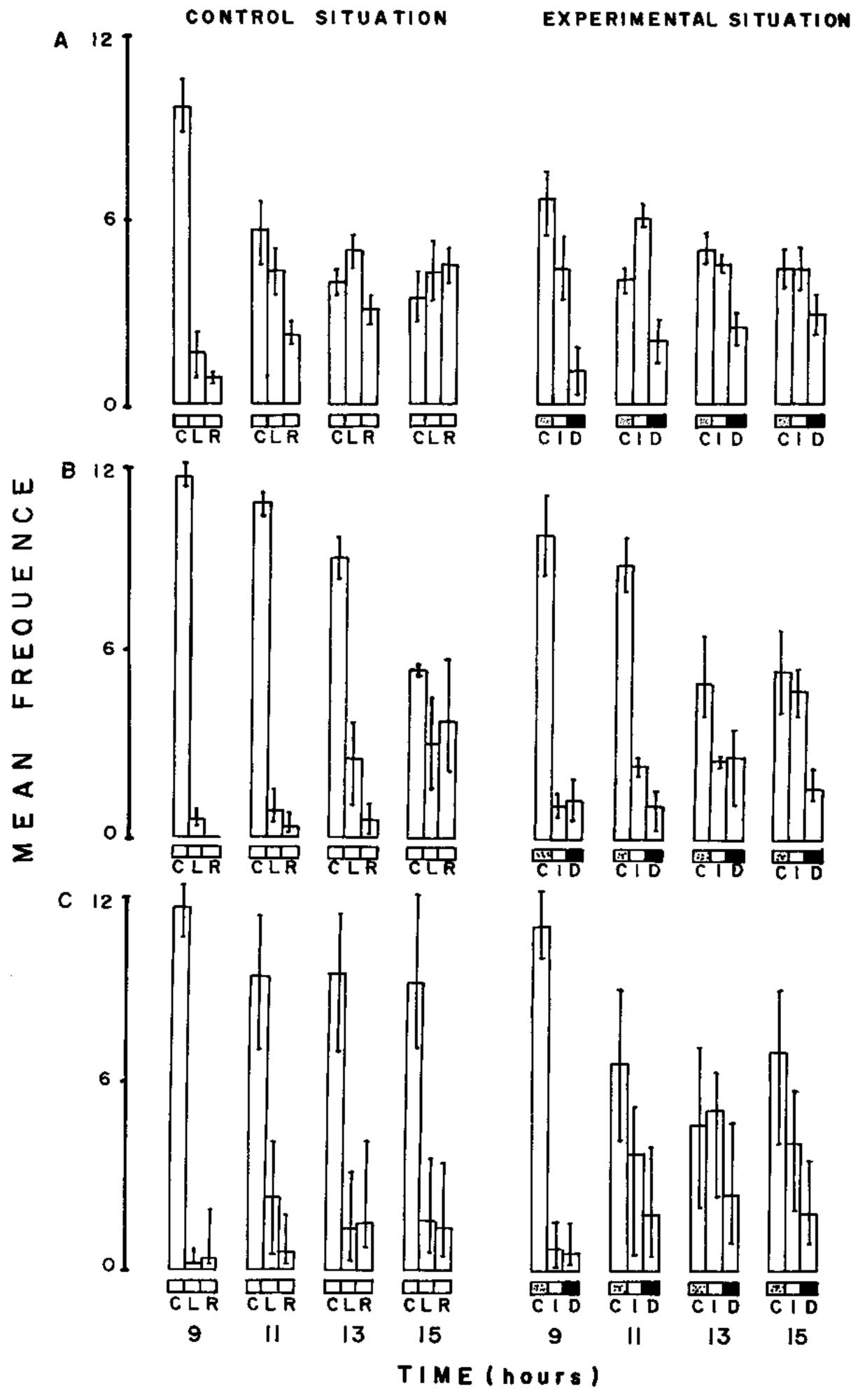


Fig. 1: mean frequence (per animal, per hour) of *B. straminea* in the arm of Y-shaped aqualrium. C (central arm); L (left arm; R (right arm); I (illuminated arm), and D (dark arm) in the Control Situation and Experimental Situation. A (Sample 1); B (Sample 2) and C (Sample 3).

The two remaining CS and ES were studied as a whole (Sample 2) because they differed from the preceding ones and showed similarities with each other in terms of frequency of localization, time and locomotion through the arms, and intragroup coefficient of variation.

Using independent sample treatment, the data for the 12 animals tested separately in CS and the 12 animals tested in ES were pooled together (Sample 3).

Effect of light on animal localization; animal localization in the Y-shaped aquarium was analyzed comparatively in CS (homogeneous lighting) and in ES (light falling on a single arm of aquarium).

Recording the position of the animals in the areas of aquarium every five minutes for each of the four daily observation periods permitted us to quantify the "frequency of animals per arm" (central, left, and right) (Fig. 1 a, b and c).

Sample 1: in CS, growing distribution towards the side arms was observed, with no significant difference between arms by the t test (Fig. 1) during the last observation period (3:00 p.m. – Fig. 1a). During the previous time periods (11 and 13 hours), the animals were more frequently found on the left side, with significant differences by the t test (Fig. 1).

In ES, the lighted arm showed the highest frequencies from the first hour of the experiment, with significant differences between this arm and the dark arm for the first three time periods (9, 11 and 13 hours). The differences were nonsignificant only for the last time period (3:00 p.m.) although the frequency was higher in the lighted arm (t test, Fig. 1).

Sample 2: both in CS and ES, the animals were found most frequently in the central arm at all time periods, with a certain distribution among the three arms observed only during the last time period (Fig. 1b).

In CS, comparison of the frequency in the two side arms revealed significant differences, with a predominance of the left arm for the first three periods of observation (9, 11 and 13 hours) and equivalent values for the last time period (15 hours, χ^2 test, Fig. 1).

In ES, the lighted arm showed significantly higher frequencies than the dark arm for the second and fourth periods of observation (11 and 15 hours) and no significant difference for the first and third periods (9 and 13 hours; X^2 test; Fig. 1b).

Sample 3 — isolated animals: in CS, the mean value obtained from the data for 12 animals tested separately revealed a high percentage of permanence in the central arm of the aquarium during all periods of observation (9, 11, 13 and 15 hours; Fig. 1c). The frequencies for the side arms were low (20% at most) and similar for the left and right side, excepting during the 2nd hour of observation, when the values for the left arm were significantly higher than for the right arm (11 hours, X^2 test; Fig. 1c).

In ES, mean frequency of localization in the arms of the aquarium for the 12 animals tested separately (six in LES and six in RES) was higher in the side arms (more than 50%) than observed in CS (about 20%). This difference demonstrates that, under heterogeneous lighting (ES), the isolated animal explores more frequently the more distal areas of the aquarium.

The frequency was higher in the lighted arm than in the dark arm at all time periods, with significant differences at 11, 13 and 15 hours (χ^2 test; Fig. 1c).

Time of exploration of the side arms in relation to the stimulus: for each sample, we measured the time of permanence in the central arm up to the time when the animal first reached the side arms of the aquarium, starting exploration.

For Sample 1, when the mean values obtained in CS (2.25 hours) are compared with those obtained in ES (1.06 hours), it can be seen that in the presence of experimental lighting (ES) exploration of the side arms took half the time to start, a significant difference at the 5% level $(\chi^2 = 29.6; d.f. = 1, p < 0.001)$.

The results obtained for Sample 2 were similar to those for Sample 3 (isolated animals) and both differed sharply from those obtained for Sample 1. In CS, mean time was 4.34 hours for Sample 2 and 5.00 hours for Sample 3, i.e. approximately double the time obtained for Sample 1 in CS. In ES, mean time was 3.54 hours for Sample 2 and 3.21 for Sample 3, also double the value obtained for Sample 1 in ES.

We would like to point out the similar behavior shown by Sample 2 (grouped animals) and Sample 3 (isolated animals).

Variation in locomotion with respect to light: the rate of animal locomotion was measured by the distance (cm) covered per hour. Sample 1 showed a locomotion rate of 138.0 ± 22.9 cm/

hour in CS and 127.5 ± 17.4 cm/hour in ES. Samples 2 and 3 showed the following values: 56.1 ± 12.1 and 48.9 ± 13.9 cm/hour in CS, and 76.9 ± 21.8 and 88.1 ± 19.1 cm/hour in ES, respectively.

The rate of locomotion varied as a function of time of day and of experimental design (CS or ES) for the three samples studied (Fig. 3). The highest locomotion rate was obtained at 11 hours (Sample 1) and 13 hours (Samples 2 and 3) in ES. In CS, the highest locomotion rate occurred at 13 hours for Samples 1 and 2 and at 11 hours for Sample 3.

When CS and ES were compared for Sample 1, no significant differences were obtained at any observation time (Mann-Whitney U test -9:00 hours, Z = 0.9109; 11:00 hours, Z = 0.539; 13:00 hours, Z = 1.060, and 15:00 hours, Z = 1.225, p > 0.05).

For Sample 2, the locomotion rate was always higher in ES than in CS, with significant differences only at 15 hours (Mann-Whitney U test, U = 123.5; n_1 and $n_2 = 20$, p < 0.05). For Sample 3, locomotion was also higher in ES than CS, with significant differences for the nine hour time period (U = 37.0, n_1 and $n_2 = 12$, p = 0.05).

When the locomotion rates of the three samples are analyzed comparatively for all time periods as a whole, significant differences are obtained for CS (Kruskal-Wallis test, H=7.73, p<0.05) and ES (H=7.53, p<0.05). The multiple comparison test was used to determine which samples differed significantly amongst themselves (Dunn procedure – Hollander & Wolf, 1973). The results showed that Samples 2 and 3 did not differ significantly in terms of locomotion rate either in CS or ES (Table I), whereas Sample 1, whose values were always higher than for the other two samples, differed significantly from Sample 3 in CS and from Sample 2 in ES (Table I).

Statistic teste (Kruskal-Wallis – H – and Dunn procedure) between the locomotion rates (cm/h) for Samples 1, 2 and 3 in the Control Situation (CS) and Experimental Situation (ES) in four hours of experiment

Situation		CS		ES			
Hour Sample	1	2	3	1	2	3	
9	109.0	58.0	31.5	139.0	53.5	60.5	
11	139.0	41.0	65.5	144.0	63.0	92.0	
13	165.0	71.0	48.0	121.0	96.0	104.0	
15	139.0	54.0	50.5	106.0	95.0	95.5	
H (1 x 2 x 3)		7.73*			7.53*		
Dunn Procedure	(1 x 2)	5.25			6.5*		
	(2×3)	1.5	1.0	1.0			
	(1×3)	6.75*		5.5			

Obs.: (p < 0.05)

The direction of locomotion was also studied for the three samples during the first period of observation (9 to 10 hours) to determine the nature of the orientation of these animals with respect to light. Table II shows that most of the animals which left the central arm during the 1st hour of the experiment (77.5% of Sample 1, 30% for Sample 2, and 25% for Sample 3) moved directly toward the stimulus, i.e. towards the lighted arm of the aquarium.

The percentage of animals which move directly towards the stimulus was greater in Sample 1 (87.1%) than in Samples 2 and 3 (67% and 66.6%, respectively). This high rate of locomotion by directional, undeviating movements towards light could suggest a kind of taxis according to the classification of Fraenkel & Cunn (1961).

Number (N) and percentage (%) of snails that left the central arm (CA) and of snails that moved directly towards the illuminated arm (AI) in the Experimental Situation.

Groups	Sample 1					Sample 2			Sample 3
	10	29	30	49	Т	1ó	29	Т	Isolated
N Total	10	10	10	10	40	10	10	20	10
N left the CA	10	6	9	6	31	2	4	6	3
N moved to IA	9	6	7	5	27	2	2	4	2
% b/a	100	60	90	60	77.5	20	40	30	25
% c/b	90	100	77	83.3	87.1	100	50	67	66.6

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 results provide evidence that the B. straminea sample studied responds positively to the light intensities tested and reveal other behavioral characteristics such as: a) a greater locomotion rate and therefore a shorter time to approach the light source than observed in B. glabrata (whose rate was 35% lower) and B. tenagophila (whose rate was 51% lower) under similar conditions (Schall, Jurberg & Vasconcellos, 1985, 1986); b) a significant tendency to move towards the left side of the aquarium under homogeneous lighting conditions (control situation); c) a lower locomotion rate for the isolated individuals in relation to 68% of those studied as groups; d) occurrence of a segment (32%) of animals tested in groups whose behavior was similar to that of the animals tested separately and different from that of the remaining animals tested in groups.

The positive response to light indicates the animal's ability to detect the stimulus and to orient towards it by a direct movement, which was detected in the area of transition between light and dark at the confluence of the aquarium, where the animal's option was direct, without deviation, as observed in *B. glabrata* (Schall, Jurberg & Vasconcellos, 1985). *B. straminea* however, even though it first moved directly towards the lit side of the aquarium, did not remain in that region, i.e. light was not a sufficient stimulus to retain it, but the animal returned to the dark areas of the unlit side arm or to the areas of the central arm, a fact that characterizes a high power for exploration of the environment on the part of this animal.

Another aspect to be considered concerns the social factors which may interfere with the response to light of the animals studied as a group. The animals are not only attracted to a favorable site but also attract each other (Eibl-Eibesfeldt, 1974). Thus, most living organisms, under natural conditions, tend to show aggregated or grouped distribution (Southwood, 1966). As far as Biomphalaria spp. are concerned, these animals have been found to form clusters both in nature and in rearing aquaria at the laboratory. Furthermore, even though these planorbids are hermaphrodites, it has been proved that cross-fertilization is preferred by animals in groups (Paraense, 1955). Simpson, Thomas & Townsend (1973) measured the level of sociability and the extension of the individual active space (area around the snail in which a behavioral response can be elicited by another individual of the same species) of B. glabrata and proved that this species is highly sociable despite an active space of small dimensions, which may be increased by mucus trails. These authors assume that these interactions may be mediated by chemical and tactile receptors and not by vision. A review by Croll (1983) has demonstrated the influence of chemoreception in the social and mating behavior of grastropods. The author cites different lines of experimental evidence which have permitted to identify chemical substances (probably pheromones) mediatin mating and aggregation in many species such as Aplysia californica (Audersirk & Audersirk, 1977); Littorina (Dinter, 1974) and B. glabrata (Townsend, 1974; Bousfield et al., 1981). Uhazy, Tanaka & Maccinis (1978) demonstrated that a water solution containing a lyophilized Biomphalaria extract is an attractant for other individuals of the same species, indicating the possible existence of a pheromone for measuring the clustering process and that proline appears to stimulate the reproductive activity of Biomphalaria. Thomas et al. (1980) contest this effect of proline and present evidence that propionic acid, a constituent of the mucus secreted by glands in the animal's sole, may act as a pheromone at diluted concentrations of 5 x 10⁻⁷ M. Other lines of evidence have shown that Biomphalaria orients itself towards water containing chemical stimulants both in the field and in

the laboratory (Bousfield, 1978; 1979) and that chemical orientation occurs through mechanisms such as clinotaxis and tropotaxis (Townsend, 1973). Similar reactions may probably occur in B. straminea, and therefore the process of social attraction should be considered to interfere with the measurement of the response to light. Taking these factors into consideration, we studied isolated animals in the aquarium to assess the action of the stimulus without social interference, in a previously cleaned environment. The results obtained for these animals proved that the significantly higher frequencies in the lighted areas are due to the mechanism of positive light orientation regardless of any social facilitation or interference, although the response of grouped animals to light was more rapid.

According to Willows (1973), under normal conditions most gastropods move in search of food and mating or to escape noxious stimuli. In the animals studied separately, the absence of the mating stimulus may have accounted, at least in part, for the lower locomotion observed in relation to the individuals studied in groups.

The greater locomotion of B. straminea in relation to two other close species, B. glabrata and B. tenagophila, studied under similar conditions, may be one of the behavioral features related to its wide distribution throughout Brazil, as indicated by the data reported by Paraense (1970). Furthermore, there is evidence that B. straminea is able to dislocate and replace B. glabrata in the field (Barbosa, 1973) and in the laboratory (Barbosa, Pereira da Costa & Arruda, 1981; 1984). Michelson & Dubois (1979) demonstrated that B. straminea is superior to B. glabrata in terms of egg hatching and has greater ability to migrate between two compartments, among other factors.

Two species compete when they use the same resource, so that any additional and persistent advantage obtained by one of them in the use of resources such as better efficiency in obtaining food, better utilization of food or greater resistance to lack of food, better ability to occupy space, or better offensive power etc., after a certain period of time may lead to the elimination of the other species when the resources are limited. Or else, under changing environmental conditions, a species gains ground during certain times, whereas at other time the other species increases without either of them being eliminated.

According to Paraense (1970), two of these species are rarely encountered in the same biotope, with little evidence of dislocating processes between them. So far only the occurrence of replacement of authoctonous *B. tenagophila* by *B. glabrata* has been reported in Manguinhos, and the converse in Belo Horizonte.

On the basis of the above considerations, two hypothesis can be raised: a) either B. straminea has a better ability to occupy spaces, with the potential capacity of fixing itself in areas previously occupied by B. glabrata, since small advances in the range of territorial occupation may represent a notable advantage in competing among migrating species (Margalef, 1980), or b) in the Northeast, where the process of replacement is being recorded, the physical and/or biological conditions of the environment may favour better acclimatization of B. straminea than of B. glabrata.

In the present study, we also noted a significant tendency of *B. straminea* to deviate towards the left side of the aquarium under conditions of homogeneous thermal and light stimulation (control situation). This was also observed in *B. glabrata* though a lower frequency, since *B. glabrata* demonstrated an equivalent distribution between the right and left side of the aquarium in CS (Schall, Jurberg & Vasconcellos, 1985). In contrast, *B. tenagophila* deviated significantly toward the right side (Schall, Jurberg & Vasconcellos, 1986). Further experimentation is need before any influence of magnetic fields on this behavior can be established.

RESUMO

As respostas comportamentais de *Biomphalaria straminea* em relação à luz, foram avaliadas através da localização em um ambiente de escolha em forma de Y e da taxa (cm/hora) e direção da locomoção sob iluminação homogênea (vertical) ou diferencial (horizontal) incidindo em apenas um dos eixos do aparelho. Utilizaram-se como fonte de luz, lâmpadas fluorescentes (luz-dodia), variando a iluminação de 2,8 a 350 lux. A análise dos dados demonstrou a existência de atração para a luz para os animais em um grupo ou isolados, embora os primeiros com um tempo de aproximação do estímulo 50% inferior aos isolados. A taxa de locomoção de *B. straminea* foi de 35% superior à encontrada para *B. glabrata* e 51% superior à de *B. tenagophila*, espécies estudadas sob condições semelhantes.

Os resultados são discutidos quanto a fatores sociais e distribuição geográfica das três espécies.

REFERENCES

- AUDERSIRK, T.E. & AUDERSIRK, G.J., 1977. Chemoreception in Aplysia californica. II. Eletrophysiological evidence of detection of the odor conspecifics. Comp. Biochem. Physiol., 56A: 267-270.
- BARBOSA, F.S., 1968. A note on *Biomphalaria straminea* (Dunker, 1848) from Manaus, state of Amazonas, Brazil, Rev. Soc. Bras. Med. Trop., 2:77-78.
- BARBOSA, F.S., 1973. Possible competitive displacement and evidence of hybridization between two Brazilian species of planorbid snails. *Malacologia*, 14:401-408.
- BARBOSA, F.S.; PEREIRA DA COSTA, D.P. & ARRUDA, F., 1981. New field observations on the competitive displacement between two species of planorbid snails inhabiting Northeastern Brazil. Mem. Inst. Oswaldo Cruz, 76:361-366.
- BARBOSA, F.S.; PEREIRA DA COSTA, D.P. & ARRUDA, F., 1984. Competitive interactions between species of freshwater snails. I. Laboratory studies: Ib. Comparative studies of the dispersal and the vagility capabilities of Biomphalaria glabrata and Biomphalaria straminea, Mem. Inst. Oswaldo Cruz, 79:163-167.
- BIJOU, S.N.; PETERSON, R.F. & AULT, M.H., 1968. A method to integrate descriptive and experimental field studies at the level of date empirical concepts. J. Appl. Behav. Anal. I:175-191.
- BOUSFIELD, J.D., 1978. Rheotaxis and chemoreception in the freshwater snail Biomphalaria glabrata (Say): estimation of the molecular weights of active factors. Biol. Bull., 154:361-373.
- BOUSFIELD, J.D., 1979. Plant extracts and chemically triggered positive rheotaxis in Biomphalaria glabrata (Say), snail intermediate host of Schistosoma mansoni (Sambon). J. Appl. Ecol., 16:681-690.
- BOUSFIELD, J.D.; TAIT, A.I.; THOMAS, J.D. & TOWER-JONES, D., 1981. Behavioural studies on the nature of stimuli responsible for triggering mucus trail tracking by *Biomphalaria glabrata*. *Malacol*. *Rev.*, 14:49-64.
- CHERNIN, E., 1967. Behavior of *Biomphalaria glabrata* and other snails in a thermal gradient. J. Parasitol., 53:1233-1240.
- CROLL, R.P., 1983. Gastropod chemoreception. Biol. Rev., 58:293-319.
- DINTER, I., 1974. Pheromonal behavior in the marine snail Littorina littorea Linnaeus. The Veliger, 17:37-39.
- EIBL-EIBESFELDT, I., 1974. Etologia, Ediciones Omega, Barcelona, 643 pp.
- FRAENKEL, G. & GUNN, D.L., 1961. The Orientation of Animals. New York, Dover Publications, 376 pp.
- HINDE, R.A., 1970. Animal Behaviour. A Synthesis of Ethology and Comparative Psychology. Mc Graw Hill, New York, 534 pp.
- HOLLANDER, M. & WOLF, D.A., 1973. Non parametric statistical methods. John Wiley & Sons. New York.
- HUTT, S.J. & HUTT, C., 1974. Observação direta e medida do comportamento. EPU e EDUSP, São Paulo, 279 pp.
- MARGALEF, R., 1980. Ecologia. Ediciones Omega S.A. Barcelona, 951 pp.
- MICHELSON, E.H. & DUBOIS, L., 1979. Competitive interaction between two snail hosts of Schistosoma mansoni: laboratory studies on Biomphalaria glabrata and B. straminea. Rev. Inst. Med. Trop. São Paulo, 31:246-253.
- MILLER, S., 1977. Planejamento experimental e Estatística. Zahar Editores, Rio de Janeiro, 176 pp.
- PARAENSE, W.L., 1955. Autofecundação e fecundação cruzada em Australorbis glabratus. Mem. Inst. Oswaldo Cruz, 53 (2):277-284.
- PARAENSE, W.L., 1970. Planorbídeos hospedeiros intermediários do Schistosoma mansoni. In: Cunha, A.S. (ed.). Esquistossomose mansoni. EDUSP. São Paulo, 13-30.
- PARAENSE, W.L., 1972. Fauna planorbídica do Brasil. In: Introdução à Geografia Médica do Brasil: Lacaz, C.S.; Baruzzi, R.G. & Siqueira Jr., W. (eds.), Ed. Edgard Blücher & USP, São Paulo :213-239.
- PARAENSE, W.L. & DESLANDES, N., 1955 a. Observations on the morphology of Australorbis glabratus. Mem. Inst. Oswaldo Cruz, 53:87-103.
- PIERI, O.S.; JURBERG, P., 1981. Aspectos etológicos na sobrevivência dos caramujos vetores da xistosomose ao tratamento com moluscicidas. *Mem. Inst. Oswaldo Cruz, 76*:47-55.
- PIMENTEL-SOUZA, F., 1977. Alguns aspectos do comportamento elementar do caramujo *Biomphalaria glabra-ta*. Departamento de Fisiologia e Biofísica, ICB, UFMG. M.Sc. Thesis. 75 pp.
- PIMENTEL-SOUZA, F.; FERNANDES-SOUZA, N.; SCHALL, V.T. & COELHO, P.M.Z., 1976 a. Comportamento elementar do caramujo *Biomphalaria glabrata*. I Disposição espacial da concha. *Rev. Bras. Pesq. Méd. Biol.*, 9:117-122.
- PIMENTEL-SOUZA, F.; SCHALL, V.T.; FERNANDES-SOUZA, N. & COELHO, P.M.Z., 1976b. Comportamento elementar do caramujo *Biomphalaria glabrata*. II Localização e tamanho da massa cefalopodal exposta. Rev. Bras. Pesq. Med. Biol., 9:123-127.
- PIMENTEL-SOUZA, F.; SCHALL, V.T.; SAUTNER JR., R.; BARBOSA, N.D.C.; SCHETTINO, M. & FERNAN-DES, N., 1984. Behavior of *Biomphalaria glabrata* (Gastropoda; Pulmonata) under different lighting conditions. *Can. J. Zool.*, 62:2328-2334.
- PINOTTI, M.; REY, L.; ARAGÃO, M.B. & CUNHA, A.G., 1960. Epidemiologia da esquistossomose e variação periódica das populações malacológicas em Pernambuco. Rev. Inst. Med. Trop. São Paulo, 2 :183-188.
- SCHALL, V.T.; JURBERG, P. & VASCONCELLOS, M.C., 1985. Orientation to light of juvenile and adult forms of melanic and albino populations of *Biomphalaria glabrata* (Say, 1818). *Mem. Inst. Oswaldo Cruz*, 80 (1):101-111.

- SCHALL, V.T.; JURBERG, P. & VASCONCELLOS, M.C., 1986. Behavioral responses to light by the snail Biomphalaria tenagophila (Orbigny, 1835). Rev. Brasil. Biol., 46:127-138.
- SIMPSON, A.W.; THOMAS, J.O. & TOWNSEND, C.R., 1973. Social behavior in the freshwater pulmonate snail B. glabrata (Say). Behav. Biol., 9:731-740.
- SOUTHWOOD, T.R.E., 1966. Ecological Methods. Methuen & Co. London.
- THOMAS, J.D.; ASSEFA, B.; COWLEY, C. & OFOSU-BARKO, J., 1980. Behavioural responses to amino acids and related compounds, including propionic acid by adult Biomphalaria glabrata (Say), snail hosts of Schistosoma mansoni. Comp. Biochem. Physiol., 66C:17-27.
- TOWNSEND, C.R., 1973. The food-finding orientation mechanism of Biomphalaria glabrata (Say). Animal Behaviour, 21:544-548.
- TOWNSEND, C.R., 1974. Mucus-trail following by the snail B. glabrata (Say). Animal Behaviour, 22 (1):170-177.
- UHAZY, L.S.; TANAKA, R.D. & MACINNIS, A.J., 1978. Schistosoma mansoni: identification of chemicals that attract of trap its snail vector, Biomphalaria glabrata. Science, 201:924-926.
- WILLOWS, A.O.D., 1973. Learning in gastropod mollusks. In: Invertebrate Learning. Corning, W.C.; Dyal, J.A. & Willows, A.O.D., eds., Plenum Press, New York: 187-274.