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# STUDIES ON SURVIVAL, BIOLOGICAL ACTIVITIES AND BEHAVIOR OF *BIOMPHALARIA GLABRATA*, THE HOST SNAIL OF SCHISTOSOMIASIS, SUBMITTED TO INCREASED HYDROSTATIC PRESSURE: A TECHNIQUE

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*To study changes in survival, in biological activities and behavior of planorbids submitted to increased hydrostatic pressure, we developed a technique using two transparent chambers and a hydraulic piston. The apparatus permitted renewal of the liquid medium without substantial variations in pressure, thus eliminating excretion products and maintaining the desired $O_2$ level and thereby permitting us to evaluate the effects of pressure independently of the occurrence of anoxia. Pressure was maintained without any contact of the liquid medium with compressed air, a situation which reproduced with relative fidelity what occurs in nature and assured the presence of the same amounts of gases in the two observation chambers (Control and Experimental).* Biomphalaria glabrata *was found to be able to survive at least 48 hours when submitted to 49.02 x $10^4$ Pa (equivalent to a water depth of 48.8 m), continuing to lay egg masses and showing few behavioral changes when compared with the control group.*

Key words: behavior – *Biomphalaria glabrata* – schistosomiasis – high pressure

The failure of several campaigns focusing on the chemical control of the transmission of schistosomiasis has become evident by the repopulation of breeding sites treated with molluscicides (W. H. O., 1965). The survival of only a few specimens is sufficient to repopulate a site within a short period of time (Paraense, 1972), owing to the fact that these snails are hermaphrodites and capable of self-fecundation (Brumpt, 1941). The so-called protective behaviors may play a relevant role in repopulation, by contributing to their survival (Pieri & Jurberg, 1981).

Migration to depths greater than usual could be included among these behaviors, as long as the snails are able to resist increased hydrostatic pressure as well as decreased illumination, temperature and concentration of dissolved $O_2$.

The general acceptance of the fact that planorbids normally inhabit shallow bodies of water (Paraense & Santos, 1953; Andrade, 1959; Paraense, 1972; Appleton, 1978) or the shallow marginal zones of deeper biotopes (Paraense & Santos, 1953) has frequently justified the application of molluscicides only to the surface or to the margins. However, some planorbids can reach higher depths as was reviewed by Jurberg et al. (1987).

From this it follows that there is a need to investigate, in detail, the possibility of planorbids surviving at different levels of hydrostatic pressure, by simulating different depths, and to observe the possible changes in biological activities and behavior caused by increased pressures.

The objectives of the present study are: 1) to test a technique which would permit the maintenance of planorbids under pressures equivalent to that of water at a depth of 48.8 m and 2) to investigate the survival ability, the performance of biological activities (copulation and egg-laying) and the occurrence of some behavioral patterns in *B. glabrata* submitted to this increased pressure for 48 hours.

## MATERIALS AND METHODS

*Snails:* We utilized 156 adult melanic specimens (14 to 16 m shell diameter; 0.45 ± 0.09 g weight, after 30 minutes on absorbing paper) of *Biomphalaria glabrata* from Touros, (State of Rio Grande do Norte, northeast of Brazil), maintained in our laboratory for several generations.

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*Liquid medium:* We used a mineral solution for snails SMC 1/2 (Jurberg et al., 1987) based on "SSW2" (Thomas et al., 1976).

*Equipment* (Fig. 1): Observing apparatus (Fig. 1B) – We built two chambers, each consisting of a transparent acrylic cylinder inserted between two anodized aluminum plates. Internally, the metal parts of the chambers were lined with white acrylic to facilitate observation of the snails and to prevent the aluminum from oxidizing. A nylon tube crossing the lid of the upper plate permitted the introduction of the liquid medium to the bottom of the chamber. Drainage occurred through a valve placed on the upper plate which forced the fluid to circulate throughout the chamber. The pressure inside each chamber was recorded with a manometer communicating with a hydraulic piston and with each chamber by means of valves. Hydraulic piston (Fig. 1A) – This device was used to supply the observation chambers with liquid medium at a constant maximum pressure of 49.02 x $10^4$ Pa and consisted of a transparent acrylic cylinder positioned between two plates of anodized aluminum. The lower plate was crossed by a hollow anodized aluminum stem whose upper end had a piston that could be impelled in two different manners to modify the pressure inside the cylinder: a) manually, with the aid of a handle fitted to the lower end of the stem, and b) with compressed air. In the latter case, a valve regulated the passage of air coming from a compressor or from a compressed air cylinder through a nylon tube into the stem, and then into the cylinder through a small opening in the stem. The stem passed through the middle of a cylindrical metal part located on the lower plate, thus permitting centralized dislocation of the piston. The lower plate also had a vent that let compressed air out when the piston was impelled downward. In the upper plate there was an opening for introducing the liquid medium, which was closed with a screw-on anodized aluminum cap, as well as a valve regulating the passage of water from the hydraulic piston to the observing apparatus.

*Procedure* – Before the experiment was started, we left the animals for at least one week in a container with SMC 1/2 and fresh lettuce ad libitum. The experiment was carried out in two stages: First stage: without renewal of SMC 1/2 – We performed three tests, each involving 26 snails subdivided into two groups, each group in a chamber. The first group was maintained under normal pressure conditions (group 1), whereas the second group was submitted to the 49.02 x $10^4$ Pa pressure increase (group 2).

Before each trial, we filled the hydraulic piston and the observing chambers with SMC 1/2 aerated for 10 min so that it would acquire approximately 7.5 mg/l of dissolved oxygen (Monitor II System, Beckman Instruments) at laboratory conditions of temperature (20° to 26° C) and pressure (9.804 x $10^4$ Pa). After the animals had been placed inside, we would close the chambers and fill them by compression of the hydraulic piston (Fig. 2A). Usually the chambers had to be tilted to eliminate air bubbles. The pressure inside the chamber containing group 2 was increased gradually until the manometer of the observing apparatus recorded a 49.02 x $10^4$ Pa increase above ambient pressure in the laboratory (Fig. 2b). We considered that once this pressure was reached, the snails were actually submitted to a pressure corresponding to a depth of 48.8 m.

We left the snails without food, at room temperature and with artificial lighting reproducing the day/night cycle. We observed the snails every 12 hours. Second stage: with renewal of SMC 1/2 – This stage differed from the preceding one only in terms of renewal of SMC 1/2 (Fig. 2C).

To avoid exposure of the animal to excessively low levels of dissolved oxygen, we performed preliminary tests in which we found that 13 specimens of *B. glabrata* weighing approximately 0.5 g can survive after 12 hours inside an observing chamber with a capacity of 1.8 l and containing 7.6 mg $O_2$/l SMC 1/2 at laboratory conditions of temperature and pressure. On the basis of these data, we decided that, starting on the 12th hour of the experiment, the mineral solution should be renewed at the end of each observing session by introducing 3.6 l of SMC 1/2 aerated for 10 minutes.

At both stages we observed the number of survivors for each 12 hours, biological activities (copulation and ovipositions) and the following behavioral patterns:

*Location* – We subdivided the observing chambers into three parts to facilitate recording animal location: 1) upper (ceiling and upperi third part of the wall); 2) intermediate; 3) low-

er part (lower third part of the wall and bottom).

*Occurrence of locomotion in general* – We recorded the number of snails moving and the number of snails not moving.

*Occurrence of behavioral patterns of locomotion* – We observed two patterns of locomotion; sliding and creeping; already described by Jurberg et al. (1987).

*Exposure of the cephalopodal mass* – To facilitate observation and to obtain better agreement among different observers, we considered the exposure and distension of the cephalopodal mass as a whole, representing a single category divided into three subcategories: 100% – cephalopodal mass totally visible and distended; 50% – cephalopodal mass partially or totally visible but contracted; 0% – cephalopodal mass inside the shell.

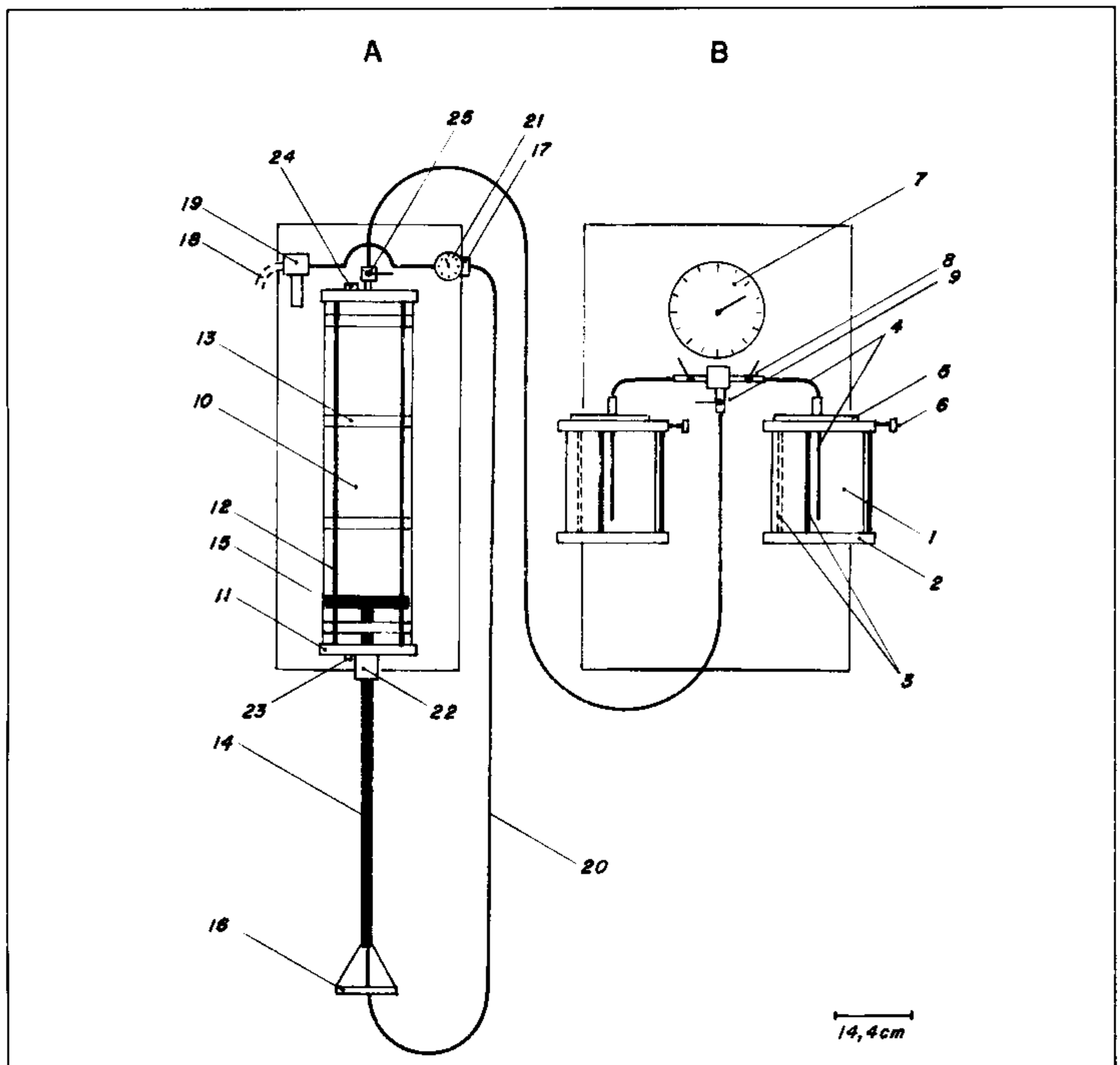


Fig. 1: Schematic representation of the apparata (numbering is according to the order of appearance of the parts of the equipament in the text. A – Hydraulic piston: 10. acrylic cylinder; 11. anodized aluminum plates; 12. aluminum rods; 13. aluminum belts; 14. hollow aluminum stem; 15. piston; 16. piston handle; 17. valve that regulates compressed air passage; 18. rubber tube that conductes air from a compressor or from a compressed air cylinder to the apparatus; 19. air filter; 20. nylon tube; 21. manometer; 22. cylindrical metal part that permits centralized dislocation of the piston; 23. vent; 24. opening; 25. valve that communicates hydraulic piston with the observing apparatus: B – Observing apparatus: 1. acrylic cylinder; 2. anodized aluminum plates; 3. aluminum rods; 4. lid of the upper plate; 5. nylon tube; 6. drainage valve; 7. manometer; 8. valve that communicate the manometer with the chambers; 9. valve that communicates the observing apparatus with the hydraulic piston.

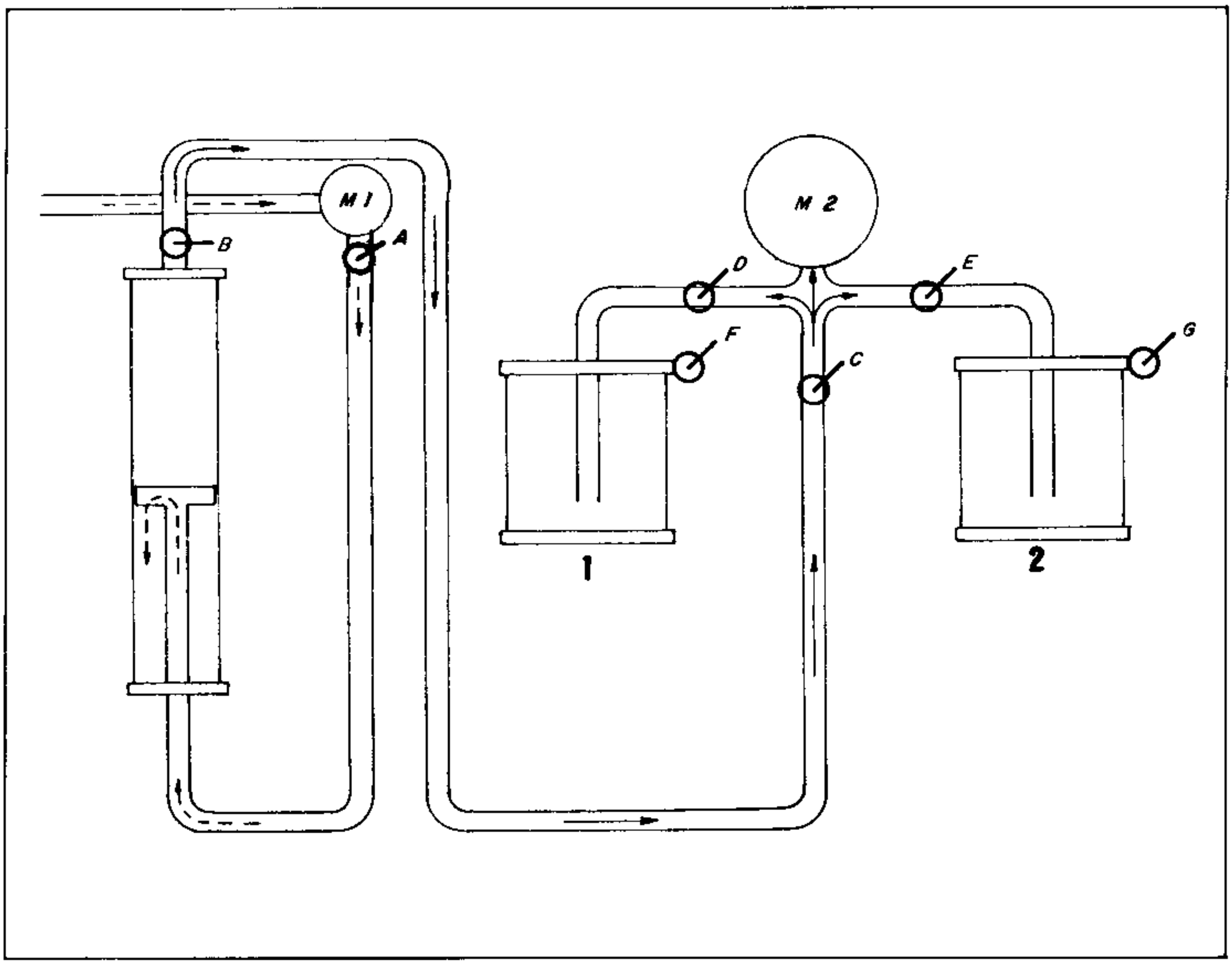


Fig. 2: Schematic representation of the situation in which the equipment was utilized (figure not in scale) 1. Fulfilling of the chambers. a. chamber 1: Valves A, B, C, D and F opened and valves E and G closed; Manometers M1 and M2 registering respectively $4.902 \times 10^4$ Pa and 0.0. Pa. b. chamber 2: valves A, B, C, E and G opened and valves D and F closed; manometers M1 and M2 registering respectively $4.902 \times 10^4$ Pa and 0.0. Pa. 2. Increase of the pressure in the chamber to be submitted to $49.02 \times 10^4$ Pa (supposing it is chamber 2): valves B, C and A opened and valves D, F and G closed; valves A being opened gradually, owing manometer M2 to increase $0.9804 \times 10^4$ Pa each minute until reaching $49.02 \times 10^4$ Pa. 3. Renewal of the liquid medium. a. chamber 1: valve D closed; manometer M2 registering permanently 0.0. Pa; valve E completely opened; valve G being opened partial and gradually; b. chamber 2: valve E closed; manometer M2 registering permanently $49.02 \times 10^4$ Pa; valve F completely opened; valve D being opened partial and gradually.
---- air flow
—— water flow

We tabulated the frequencies of some behaviors of *B. glabrata*, of biological activities and survivors observed in each experimental situation for 48 hours and, when necessary, analyzed them statistically by the chi-square test for comparison of two situations (with or without renewal; with or without increased pressure) in each observing session at the 5% level of sginificance. These analyses, were carried out to verify which factor was contributing more to the changes: pressure or water renewal.

## RESULTS

*Survival* – All of the 78 animals submitted to increased hydrostatic pressure survived until the end of the experiment (Table I). A single case of death was observed in the group maintained at ambient pressure of the laboratory during the stage with renewal of SMC 1/2.

*Biological activities* – No copulations occurred during the observing sessions at any stage. The three ovipositions observed occurred when we submitted the snails to increased pressure. Two of them occurred in the group with renewal and the other in the group without renewal of SMC 1/2. It was possible to count the number of eggs only in one of the egg-masses of the group with renewal of the me-

dium (32 eggs), because of their location on the acrylic wall. The other two egg-masses occurred on the bottom of the chamber and on the shell of a snail, thus preventing the eggs from being counted.

*Behavior* – Snails location in the observing chambers (Table II) seemed to be affected more frequently by renewal of the medium than by pressure.

The frequency of locomotion (Table III) was not affected by pressure increase, but was affected by the liquid medium renewal in 5 of the 8 observing sessions (4 at each pressure).

In all groups, locomotion occurred by creeping during the 12 hours of experiment (Table IV) and practically all snails exposed 100% of the cephalopodal mass throughout the experiment (Table V).

TABLE I

Influence of renewal of liquid medium and of hydrostatic pressure increase on survival of *Biomphalaria glabrata:* frequency (F) and percentage (%) of survival after 48 hours of experiment

| Meters | With renewal of SMC 1/2 | | Without renewal of SMC 1/2 | | Total | |
|---|---|---|---|---|---|---|
| | F | % | F | % | F | % |
| 0 | 38 | 97.4 | 39 | 100 | 77 | 98.7 |
| 48.8 | 39 | 100 | 39 | 100 | 78 | 100 |
| Total | 77 | 98.7 | 78 | 100 | | |

TABLE II

Influence of renewal of liquid medium and of hydrostatic pressure increase on *Biomphalaria glabrata* snails location in observing chambers

| Hours | Location | Number of snails | | | | Results of chi-square tests | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Effect of water renewal | | Effect of pressure increase | |
| | | With renewal | | Without renewal | | | | | |
| | | 0 m | 48.8 m | 0 m | 48.8 m | 0 m | 48.8 m | With renewal | Without renewal |
| | S | 21 | 13 | 6 | 12 | | | | |
| 12 | M | 3 | 4 | 8 | 4 | 12.672* | 0.062 | 3.350 | 3.342 |
| | I | 15 | 22 | 24 | 23 | | | | |
| | S | 21 | 8 | 18 | 17 | | | | |
| 24 | M | 1 | 5 | 2 | 3 | 0.580 | 4.829 | 10.378* | 0.243 |
| | I | 17 | 26 | 18 | 19 | | | | |
| | S | 12 | 16 | 22 | 23 | | | | |
| 36 | M | 1 | 0 | 0 | 3 | 15.041* | 5.630 | 2.233 | 3.900 |
| | I | 26 | 20 | 6 | 13 | | | | |
| | S | 12 | 6 | 24 | 20 | | | | |
| 48 | M | 1 | 0 | 3 | 4 | 27.072* | 18.289* | 3.830 | 1.109 |
| | I | 26 | 33 | 11 | 15 | | | | |

S = superior part; M = middle part; I = inferior part; * = significant difference (2 degrees of freedom, 0.05 significance level).

TABLE III

Influence of renewal of liquid medium and of hydrostatic pressure increase on frequence of *Biomphalaria glabrata* snails locomotion

| Hours | Location | Number of snails | | | | Results of chi-square tests | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Effect of water renewal | | Effect of pressure increase | |
| | | With renewal | | Without renewal | | 0 m | 48.8 m | With renewal | Without renewal |
| | | 0 m | 48.8 m | 0 m | 48.8 m | | | | |
| 12 | M | 17 | 14 | 20 | 27 | 0.630 | 8.690* | 2.230 | 0.482 |
| | NM | 22 | 25 | 18 | 12 | | | | |
| 24 | M | 19 | 19 | 16 | 25 | 0.338 | 1.877 | 3.741 | 0.000 |
| | NM | 20 | 20 | 22 | 14 | | | | |
| 36 | M | 5 | 5 | 21 | 14 | 15.557* | 5.636* | 2.911 | 0.000 |
| | NM | 34 | 34 | 17 | 25 | | | | |
| 48 | M | 2 | 1 | 16 | 9 | 14.705* | 7.933* | 3.178 | 0.347 |
| | NM | 37 | 38 | 22 | 30 | | | | |

M = moving; NM = not moving; * = significant difference (2 degrees of freedom, 0.05 significance level).

TABLE IV

Frequency of occurrence of each locomotion pattern of *Biomphalaria glabrata*

| Hours | Patterns | 0 m | | 48.8 m | |
|---|---|---|---|---|---|
| | | With renewal | Without renewal | With renewal | Without renewal |
| 12 | creeping | 14 | 15 | 22 | 26 |
| | sliding | 2 | 5 | 0 | 0 |
| 24 | creeping | 18 | 14 | 16 | 22 |
| | sliding | 0 | 2 | 0 | 0 |
| 36 | creeping | 5 | 19 | 9 | 16 |
| | sliding | 1 | 2 | 1 | 0 |
| 48 | creeping | 5 | 16 | 1 | 9 |
| | sliding | 0 | 0 | 0 | 0 |

TABLE V

Frequency of each percentage of exposure of the cephalopodal mass of *Biomphalaria glabrata*

| Hours | Percentage | 0 m | | 48.8 m | |
|---|---|---|---|---|---|
| | | With renewal | Without renewal | With renewal | Without renewal |
| 24 | 0 | 0 | 0 | 0 | 0 |
| | 50 | 0 | 0 | 0 | 0 |
| | 100 | 39 | 38 | 39 | 39 |
| 24 | 0 | 0 | 0 | 1 | 0 |
| | 50 | 1 | 0 | 2 | 0 |
| | 100 | 38 | 38 | 36 | 39 |
| 36 | 0 | 0 | 0 | 2 | 0 |
| | 50 | 0 | 0 | 1 | 0 |
| | 100 | 39 | 38 | 36 | 39 |
| 48 | 0 | 0 | 2 | 1 | 2 |
| | 50 | 0 | 0 | 0 | 1 |
| | 100 | 39 | 36 | 38 | 36 |

## DISCUSSION AND CONCLUSIONS

The apparatus presented in this study is effective to observe adult Biomphalaria snails behavior at pressures up to that corresponding to 48.8 m of water depth. It permitted renewal of the liquid medium without substantial variations in pressure, thus eliminating excretion products and maintaining the desired $O_2$ level and thereby permitting us to evaluate the effects of pressure independently of the occurrence of anoxia. It was possible to maintain pressure without any contact of the liquid medium with compressed air, a situation wich reproduced with relative fidelity what occurs in nature, and assured the presence of the same amounts of gases in the two observation chambers.

The development of special pressure chambers has permitted the study of deep-sea organisms, such as zooplancton (McDonald & Gilchrist, 1969), amphipods (Yayanos, 1978; 1981; McDonald & Gilchrist, 1982), and small groups of Crustacea, submitted to pressures of more than "100 atm" ($1013 \times 10^4$ Pa). Blaxter & Tytler (1972 according to McDonald 1975) maintained fish at pressures up to $202.6 \times 10^4$ Pa. Sommer (1981) subjected barnacles to pressures corresponding to a depth of 20 m. Dale (1984) standardized a method for maintaining macrophytes at a pressure corresponding to a depth of 23 m. Nojima & Sato (1981) maintained egg-masses of *Biomphalaria glabrata, B. pfeifferi* and *Bulinus globosus* in a syringe, subjecting them to a maximum pressure of "4 atm" ($40.52 \times 10^4$ Pa).

However, none of these would be apparatuses fully satisfactory for the observation of adult planorbids submitted to pressures corresponding to those occurring at the bottom of dams. Thus, the technique described by Sommer (1981) would necessitate a 50 m height column to add $49.0 \times 10^4$ Pa. The apparatuses described by Nojima & Sato (1981) and Dale (1984) maintained pressure through direct contact of the water with compressed air, and did not allow water renewal at constant pressure to eliminate excreta. The technique of Dale (1984) had the advantage of, at least, permitting $O_2$ renewal. The dimensions of the apparatus used by McDonald & Gilchrist (1969, 1982) and Nojima & Sato (1981) are incompatible with the size and mobility of adult planorbids. Except for the pressure chambers described by Dale (1984), no equipment, according to the descriptions, appears to have a sufficiently extensive transparent surface to permit accurate observation of the snails.

The 100% survival of the snails subjected to increased hydrostatic pressure for 48 hours in the present study, points to the need to evaluate in detail the possibility that *B. glabrata* can live and occasionally form populations at depths greater than usual, thus being able to repopulate breeding sites.

It is important to call attention to the possibility of snails surviving at the bottom of dams. Although it is not frequent to find dissolved oxygen at high depths, we must consider that *B. glabrata* is able to live without $O_2$ during 16 hours (Von Brandt et al., 1948) and that some lakes such as Don Helvecio lake at Rio Doce Valley, (Mitamura & Hino, 1985) have dissolved oxygen (3.86 mg/l) at the bottom (22 m).

To investigate in detail the survival ability, biological activities and behavior of *Biomphalaria glabrata* subjected to increased hydrostatic pressure, it is necessary to perform long-lasting studies under different conditions of lighting, temperature, feeding and dissolved $O_2$, $CO_2$ and $N_2$ levels, in order to simulate as accurately as possible the conditions present at great depths in planorbid breeding sites. Adequate levels of lighting and temperature can be obtained as long as the apparatus is maintained in a place with controlled conditions.

To study the effects of pressure in an environment in which anoxia and the accumulation of toxic wastes will not be critical, the liquid medium must be circulated without any substantial changes in pressure, since, as shown by statistical analysis, the behaviors studied during 48 hours of experiment were often affected not only by increased pressure, but also and mainly by maintaining the snails without renewal of the liquid medium. By using the present technique, which permitted renewal of the medium without great changes in pressure, it is possible to approach ideal conditions by simply reducing the interval between renewals.

Maintaining pressure with no contact between the liquid medium and compressed air, in addition to reproducing more faithfully what occurs in nature, guarantees an identical gas

supply to the two observing chambers, so that the effect of pressure can be investigated separately with no interference from the oxygen supply. However, fundamental differences exist between the two chambers concerning the ease with which the animals in each group can take in $O_2$ and give off $CO_2$ into the medium.

This technique is limited in terms of the possibility of supplying food to the animals, since food should be renewed periodically to prevent it from decaying without significant changes in pressure. Under the present conditions, in order to perform experiments without food deprivation it is strictly necessary to utilize germ-free animals, sterile liquid medium and decontaminated food. These will remain in good condition until the end of the experiment.

The fact that no copulation was observed does not exclude the possibility of copulations having occurred during the intervals between observing sessions.

It is premature to assume that the egg-masses observed were expelled from the genital ducts because of the increased pressure, even though only animals submitted to $49.02 \times 10^4$ Pa. Pressure laid eggs, because the reduced number of ovipositions did not permit reaching any conclusion in this respect. Since temperature was in the range compatible with the survival and reproduction of *B. glabrata* (Brumpt, 1941; Paraense, 1972) and prolonged immersion does not prevent oviposition (Jurberg et al., 1982), food deprivation, which lasted throughout the experiment, probably affected oviposition in both groups. Furthermore, preliminary tests have demonstrated that the apparatus does not prevent oviposition, since animals provided with fresh lettuce lay eggs at the observing chambers when these are opened (animals with free access to the surface).

The possibility of counting eggs and observing embryo development can be considered fairly good, depending on the location of the egg-masses.

These results indicate the need to continue the investigations on planorbids under different levels of hydrostatic pressure, in different conditions of food supply, temperature and concentration of dissolved $O_2$.

RESUMO

**Estudos sobre a sobrevivência, atividades biológicas e comportamento de *Biomphalaria glabrata* hospedeiro intermediário da esquistossomose, submetido a aumento da pressão hidrostática: uma técnica** – Para estudar mudanças na sobrevivência, atividades biológicas e comportamento de planorbídeos submetidos a aumento de pressão hidrostática, desenvolvemos uma técnica que utiliza duas câmaras transparentes e um pistão hidráulico. O aparelho permitiu a renovação do meio líquido sem variações substanciais na pressão, eliminando assim os produtos de excreção e mantendo o nível de $O_2$ dissolvido desejado, e desse modo permitindo-nos avaliar o efeito da pressão independente da ocorrência de anoxia.

A pressão foi mantida sem nenhum contato do meio líquido com o ar comprimido, situação que reproduziu com relativa fidelidade o que ocorre na natureza, e assegurou a presença da mesma quantidade de gases nas duas câmaras de observação.

*Biomphalaria glabrata* foi capaz de sobreviver pelo menos 48 horas quando submetida a $49{,}02 \times 10^4$ Pa (equivalente a 48 m de profundidade) continuando a pôr massas ovíferas, e mostrando poucas modificações comportamentais quando comparada com o grupo de controle.

Palavras-chave: comportamento – *Biomphalaria glabrata* – xistosomose

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REFERENCES

ANDRADE, R. M., 1959. Ecologia. *Rev. Brasil. Mal. D. Trop., 11* (273): 171-217.

APPLETON, C. C., 1978. Review of literature on abiotic factors influencing the distribution and life cycle of bilharziasis intermediate host snails. *Malacological Review, 11:* 1-25.

BLAXTER, J. H. S. & TYTLER, P., 1972. Pressure discrimination in teleost fish. *Symp. Soc. Exp. Biol., 26:* 417-443.

BRUMPT, E., 1941. Observations biologiques diverses concernant *Planorbis (Australorbis) glabratus,* hote

intermediaire de *Schistosoma mansoni. Ann. Parasitol., 18* (1, 2, 3): 9-45.

DALE, H. M., 1984. Hydrostatic pressure and aquatic plant growth: a laboratory study. *Hydrobiologia, 111:* 193-200.

JURBERG, P.; GATTI, M. J.; SOARES, M. S. & BARBOSA, J. V. 1982. Comportamento e condições de sobrevivência de *Biomphalaria glabrata* submetida a imersão prolongada. *Ci. e Cult. Resumos, 34* (7): 802.

JURBERG, P.; SCHALL, V. T.; BARBOSA, J. V.; GATTI, M. J. A. & SOARES, M. S., 1987. Behavior of *Biomphalaria glabrata,* the intermediate host snail of *Schistosoma mansoni,* at different depths in water in laboratory conditions. *Mem. Inst. Oswaldo Cruz, 82* (2): 197-208.

MAC DONALD, A. C., 1975. *Physiological aspects of deep sea biology.* Mongr. Physiol. Soc. Cambridge University Press, Cambridge, 450 p.

MAC DONALD, A. C. & GILCHRIST, I., 1969. Recovery of deep sea plancton at constant pressure. *Nature, 222:* 71-72.

MAC DONALD, A. C. & GILCHRIST, I., 1982. The pressure tolerance of deep sea amphipodes collected at their ambient high pressure. *Comp. Biochem. Physiol., 74A:* 349-352.

MITAMURA, O. & HINO, K., 1985. Distribution of chemical constituents of the Rio Doce Valley Lakes. In. SAIJO, Y. & TUNDISI, U. G. Limnological studies in Central Brazil, 1st. Report: 53-62.

NOJIMA, H. & SATO, A., 1981. Effect of Temperature and Atmospheric Pressure on the eggs of the intermediate snail host of *Schistosoma mansoni* and *S. hematobium. Jap. J. Parasit., 30* (2): 121-126.

PARAENSE, W. L., 1972. Fauna Planorbídica do Brasil, p. 213-239. In: C. S. Lacaz et al. *Introdução a geografia Médica do Brasil.* Eds. Blucher e Universidade de São Paulo.

PARAENSE, W. L. & SANTOS, J. M., 1953. Um ano de observação sobre Esquistossomose em planorbídeos na Lagoa Santa. *Rev. Bras. Malariol. D. Trop., 5* (3): 253-269.

PIERI, O. S. & JURBERG, P., 1981. Aspectos etológicos na sobrevivência dos caramujos vetores da xistosomose ao tratamento com moluscicidas. *Mem. Inst. Oswaldo Cruz, 26* (1): 47-55.

SOMMER, H. H., 1981. Perception of constant hydrostatic pressure. A physilogical basis for the vertical stratification of marine habitats. *Experientia, 37:* 141-143.

THOMAS, J. D., POWLES, M. & LODGE, R., 1976. The chemical Ecology of *Biomphalaria glabrata:* the effects of ammonia on the growth rate of juvenile snails. *Biol. Bull., 151:* 386-397.

VON BRANDT, T.; NOLAN, M. O. & MANN, E. R., 1948. Observations on the respiratory of *Australorbis glabratus* and some others snails. *Biol. Bull., 95:* 199-213.

WORLD HEALTH ORGANIZATION, 1965. Molluscicide screening and evaluation. *Bull. W. H. O., 33* (4): 567-581.

YAYANOS, A. A., 1978. Recovery and Maintenance of live amphipods at a pressure of 580 Bars from an ocean Depth of 5.700 meters. *Science, 200:* 1056-1059.

YAYANOS, A. A., 1981. Reversible inactivation of deep-sea amphipods *(Paralicella capresca)* by a decompression from Bars to atmospheric pressure. *Comp. Biochem. Physiol., 69A:* 563-565.