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COMPETITIVE INTERACTIONS BETWEEN SPECIES OF FRESHWATER SNAILS. I. LABORATORY: Ia. GENERAL METHODOLOGY

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For the development of laboratory experiments on the competitive interactions between freshwater snail populations, special snail rooms were set up in the main building of the Research Center "Aggeu Magalhães". In the current paper, the first of a series on this subject, the general methodology of the laboratory work is described in detail. Using indoor cement channels in which a uniform seminatural environment was created, interactions of freshwater snail populations can be studied with minimal interference of the usual variables. Controlled indoor environmental techniques, as described in the current paper, may also be utilized in different types of experiments in malacology, and represent a substantial technical advance in malacological work.

Observations made in the coastal area of the State of Pernambuco, northeastern Brazil (Barbosa, 1973), on the competitive superiority of *Biomphalaria straminea* over *B. glabrata*, led our research group to develop laboratory and field experiments to study the interactions between species of fresh water snails.

Initial contacts with the higher direction of the "Fundação Oswaldo Cruz" in Rio de Janeiro in 1974 were very encouraging. In 1976 specific funds were committed to the Research Center "Aggeu Magalhães" in Recife, institution that became responsible for carrying out a special program on snail biological control.

For the development of such experiments special snail rooms were set up. The idea was to create a uniform environment in which two snail species would interact as much as possible out of the influence of the natural, physical and some of the biological variables that would interfere with the dynamics of the snail populations.

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THE PROJECT ORGANIZATION AND OPERATION

Eight months (May-December, 1976) were spent in mounting the laboratories, training personnel and establishing severe rules for the control of the snail rooms.

Only personnel working directly with the project or accompanied visitors were admitted to the research area. All possible additional precautions were taken to avoid contamination of the snail cultures.

Under this atmosphere in May 1977 five channels were seeded with *B. glabrata* and other five with *B. straminea*.

In spite of all measures taken, two years were lost due to unsuitability of certain members of the laboratory team. This resulted in severe damage to the snail cultures.

Part of the original personnel working at the project was replaced and the new team members were trained and instructed. Channels were completely renewed.

Finally, at the end of 1979 the operational phase of the project started.

At that moment the laboratory manpower was composed of one senior research officer, two research technicians, three research auxiliaries and one servant.

THE INDOOR CHANNELS

Thirteen channels were set up in three separate rooms of the main building of the Research Center (Figs. 1 and 2).

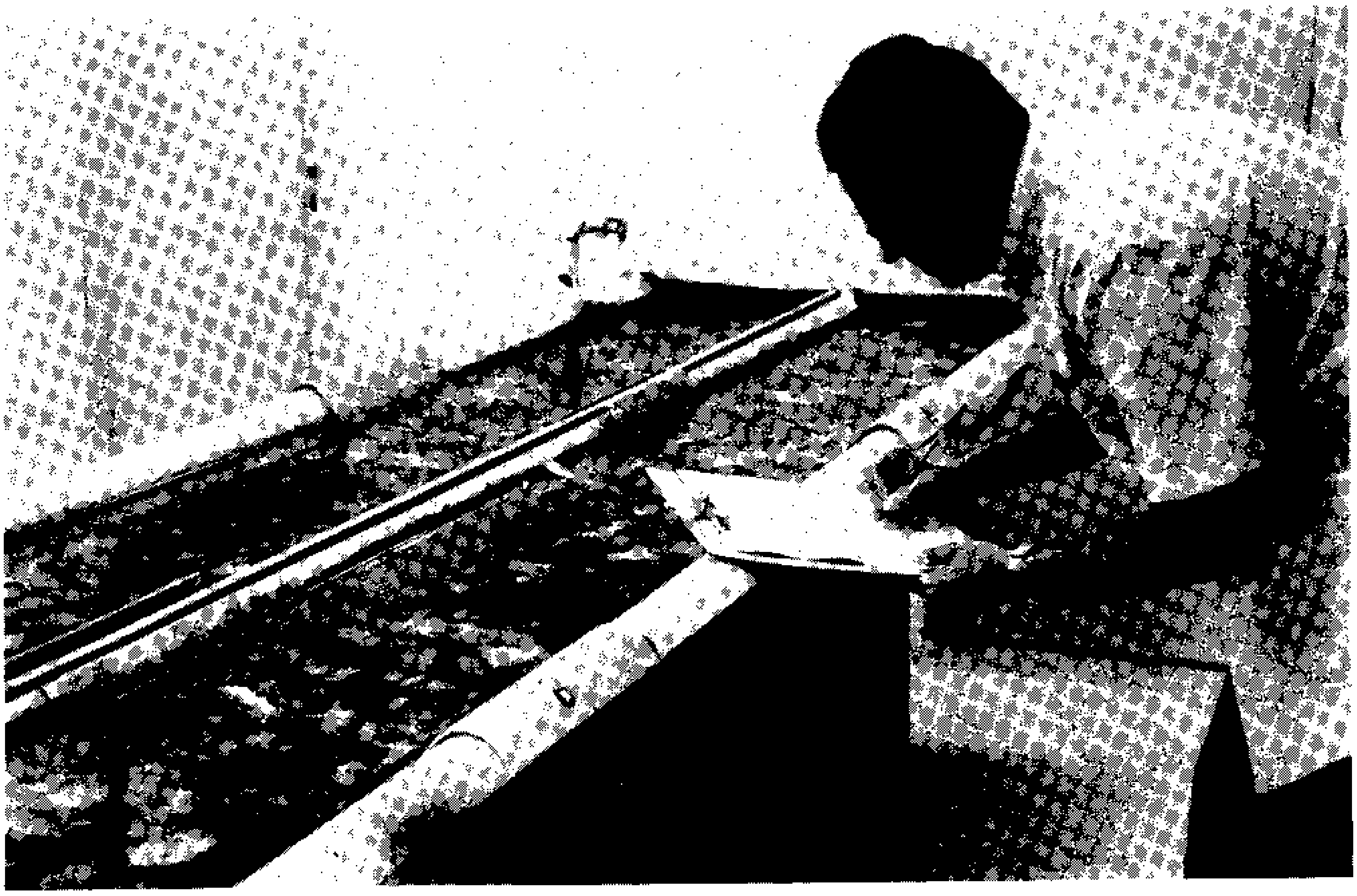

Fig. 1 – Photograph of the channels. On both sides of the research worker the emergent parts of two collecting stations are seen. They are labelled *B* (left) and *A* (right). A water tap is seen at the end of one channel.

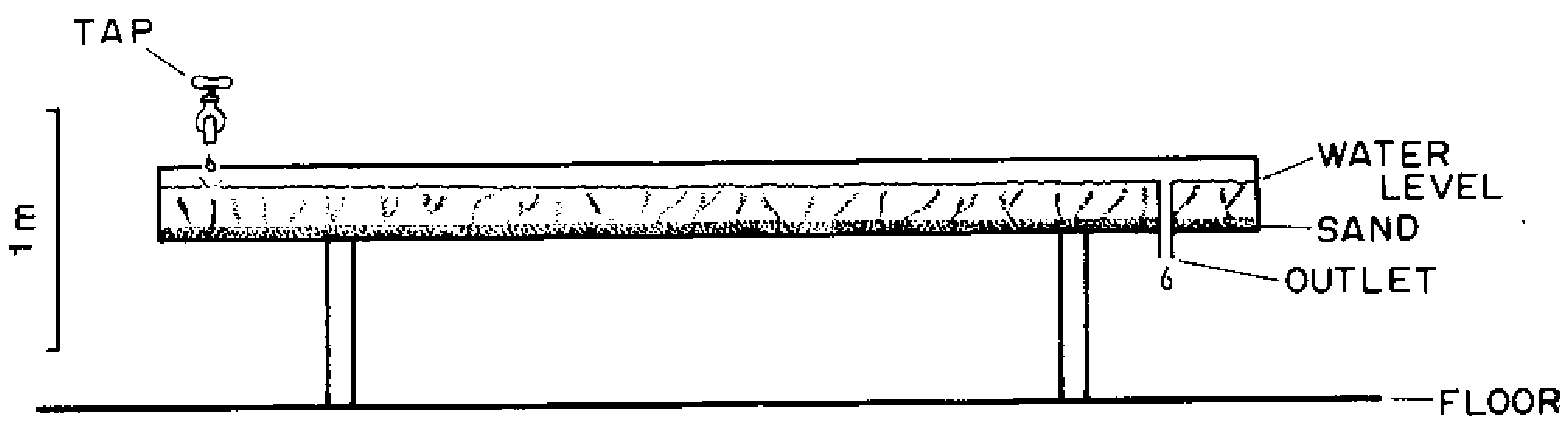

Fig. 2 – Longitudinal section of a 4.5 m long channel.

Cement roof covers of the trade mark "Kalheta" built by "Brasilit", were fixed upside down on strong supporters in such a way that the superior margin of the channel became at 95 cm over the floor. Eight channels were 4.5 meters long while the remaining five were 3.5 meters long.

Both ends of the channels were closed with cement walls. At one end of each channel a tap was fixed at 16 cm over the water surface. At the opposite end, an outlet was placed to regulate the volume of the water inside the channel.

The channels were only allowed for use after having been thoroughly brushed and washed with neutral soap or detergent for the period of 30 days. During this period the water was renewed every day.

THE SNAIL STOCKS

The target population is a laboratory-bred strain of *B. glabrata* highly susceptible to the infection with *Schistosoma mansoni* originated from natural breeding places in the county of Paulista, state of Pernambuco.

Two strains of *B. straminea* are being tested now as competitors of *B. glabrata*: *R3*, an albino strain resistant to the infection with *S. mansoni* and *PT*, an albino (black eyes) strain partially resistant to the infection. The former has been kindly offered us by Dr. C.S. Richards, from the National Institutes of Health, Bethesda, Md, USA, and was originated from Sete Lagoas, state of Minas Gerais, Brazil. The strain labelled *PT* was collected in natural breeding places of a nonendemic area, Petrolina county, backlands of the state of Pernambuco.

Several other strains of *Biomphalaria*, as well as other potential snail competitors, are being kept in the snail breeding rooms.

THE AQUATIC PLANTS

Only one species of aquatic plant, *Elodea canadensis*, was used during the experiments. Plants collected in the field are placed in outdoor large cement tanks. From there the plants, after being washed, selected, and carefully watched for snails under a hand lens, are transferred to cement screened tanks under a covered terrace. This is called first stage and has the duration of ten days. Only fresh vivid plants are used.

Second stage includes the examination of plants through a hand lens and their conservation for 20 days in small glass aquaria in the laboratory.

In the last stage, the plants are kept in 35 liter glass aquaria in the laboratory during other ten days, after having been again examined under a hand lens. Then they are considered free of snails and can be transferred to the channels or be used for any other type of laboratory work.

With careful inspection of the aquatic plants as described above it was possible to keep the channels free of snails. Only in one instance contaminant snails have been seen at the last stage. Whenever snails or eggs are seen at any stages the whole content of the tank is removed to the first stage.

Both indoor aquaria and channels received natural day light through ordinary windows. Artificial lights placed in the snail rooms to increase the amount of light was only used during cloudy dark days.

THE WATER AND SAND

Tap water is being used in the channels during the experiments. The water before arriving to the channels is dechlorinated through consecutive passages in two tanks placed at the roof of the building. The water drops continuously over the surface of the water and its input is regulated in such a way that the whole content of the channel is replaced every thirty days.

The whole volume of the water in the channels is kept constant through the above feeding mechanism, but in fact the work is practically carried out in standing water.

In the eight channels, measuring 4.5 meters, the whole volume of water varies from 137 to 142 liters. In the 3.5 meters remaining channels the whole volume of the water varies from 85 to 90 liters. The water surface measures 35 cm in width. The distance from the surface of the sand layer to the water surface is kept at 11 cm. The sand layer is on an average 3 cm thick.

The water is kept at pH neutral point by using calcium carbonate powder.

Sand used in the channels is taken from a river on the outskirts of Recife. The sand is screened and washed in the laboratory before its use.

The water temperature is kept at 22.4°C (SD 0.77) by the use of air conditioning ordinary equipment which is only used in hot days during summer.

SNAIL FEEDING, DISTRIBUTION AND REPRODUCTION

Lettuce was the everyday food for the snails. In addition, on alternate weeks, the snails were offered cooked-leaves of stinging-nettle (*Fleurya aestuans*), fresh wheat germ and fish-food powder.

Shells of dead snails are desintegrated and serve as secondary source of calcium. Snails have also been seen feeding directly on clusters of calcium carbonate.

Green algae seen covering the channel walls or floating on the water and organic matter found at the bottom of the water are the preferential food consumed by young snails.

During the two years of the observation period, the shells of snail strains breeding in the channels were always found to be with their ordinary resistant texture and their surface was never eroded as commonly seen in laboratory bred planorbid specimens.

Observations have shown that snails are apparently uniformly distributed along the channels. However, they are attracted by the food and, sometimes, around the area of water dropping.

To avoid the uneven distribution of snails in the channels snail feeding was interrupted and the water taps closed 24 hours before the snail counting procedure.

Snail eggs are more commonly found on the leaves of *Elodea canadensis* or on the undersurface of lettuce. However, snails lay their eggs on any solid surface such as the channel walls, the shell of other snail specimens, and even on the glass cover of the water thermometer or on the external surface of the water outlet.

SNAIL POPULATION DYNAMICS

The dynamics of the snail population was studied by examination, in most cases, of a 20 per cent sample taken at regular weekly intervals from the whole content of the channels.

For each channel, fixed collecting stations were established at regular intervals (Fig. 1). Each station was made of a cross section of a channel of the same size, and inserted as a lining sheet over the inner surface of the channels used in the experiments (Fig. 1, 3 and 4).

At the moment of the sampling collection, both open sides of the stations are tightly closed with wooden walls kept by means of two clamps each. To avoid escaping of very young snail specimens the wooden pieces were set in a frame of soft rubber. At that moment, the whole channel segment (collecting station) is gently pulled up while the water content leaks through screened holes placed at the lateral surface of the wooden pieces.

The whole content of the sample is then spread over a large shallow pan and the snails are counted and measured. Plants and sand are carefully watched under a low magnification stereoscope. After this operation, snails are put back in the channels. Fig. 4 shows some aspects of the work at the channels.

ORDINARY CARE OF CHANNELS

Once the channels had been prepared and seeded they were carefully watched. In fact, the channels did not require close inspection. Direct observation of the channels for the period of two years after snails had been seeded suggested the following routine measures: 1. control of temperature and pH; 2. removal of excess organic matter accumulated at the bottom of the channels; 3. renewal of decaying plants.

The above measures, however, require careful administrative organization of the research project as previously mentioned.

The usual components of the aquatic fauna, such as oligochaetes, and ostracods, are seen growing in the channels. They have been usually found in small numbers and have not interfered with the experiments.

Only once was an "epizootic" infestation of the channels by ostracods observed. This was quickly controlled by patient daily mechanical removal of this snail pest. In a very short time the ostracod population was ultimately reduced to a low level. This accident did not interfere with the growth of the snail cultures.

COMMENTS

The methodology described in the current paper has been proved extremely useful for the development of experiments on snail population interactions that are being presently carried out in our laboratory.

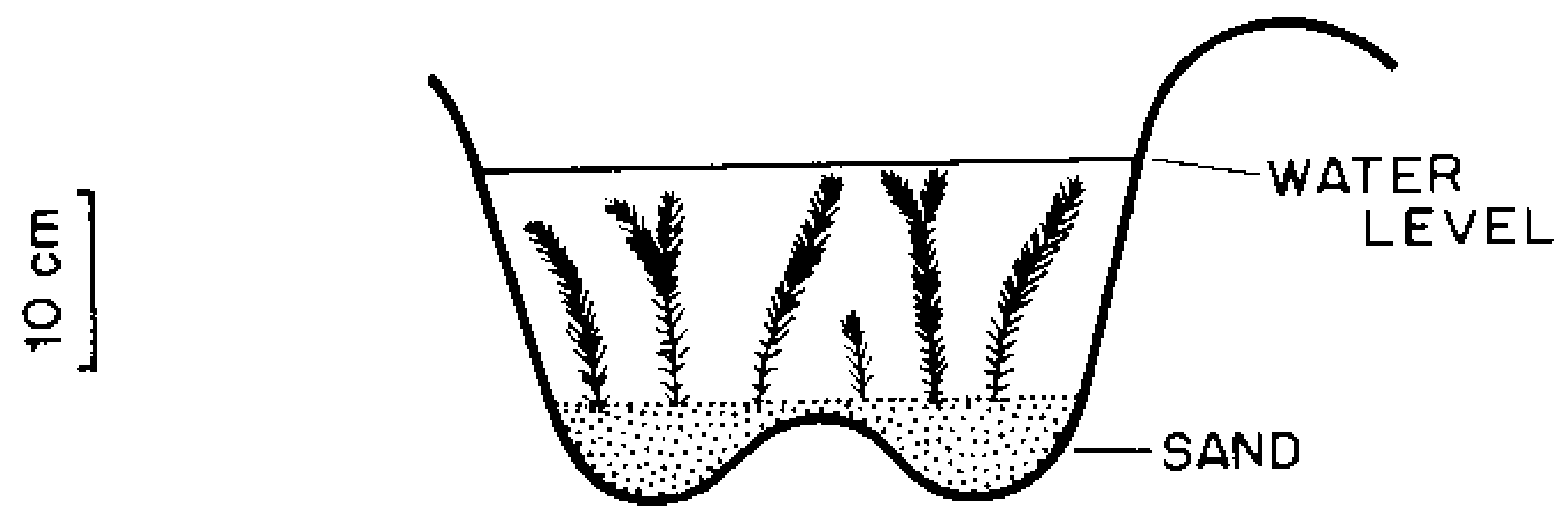

Fig. 3 – A cross section of a 4.5 m long channel.

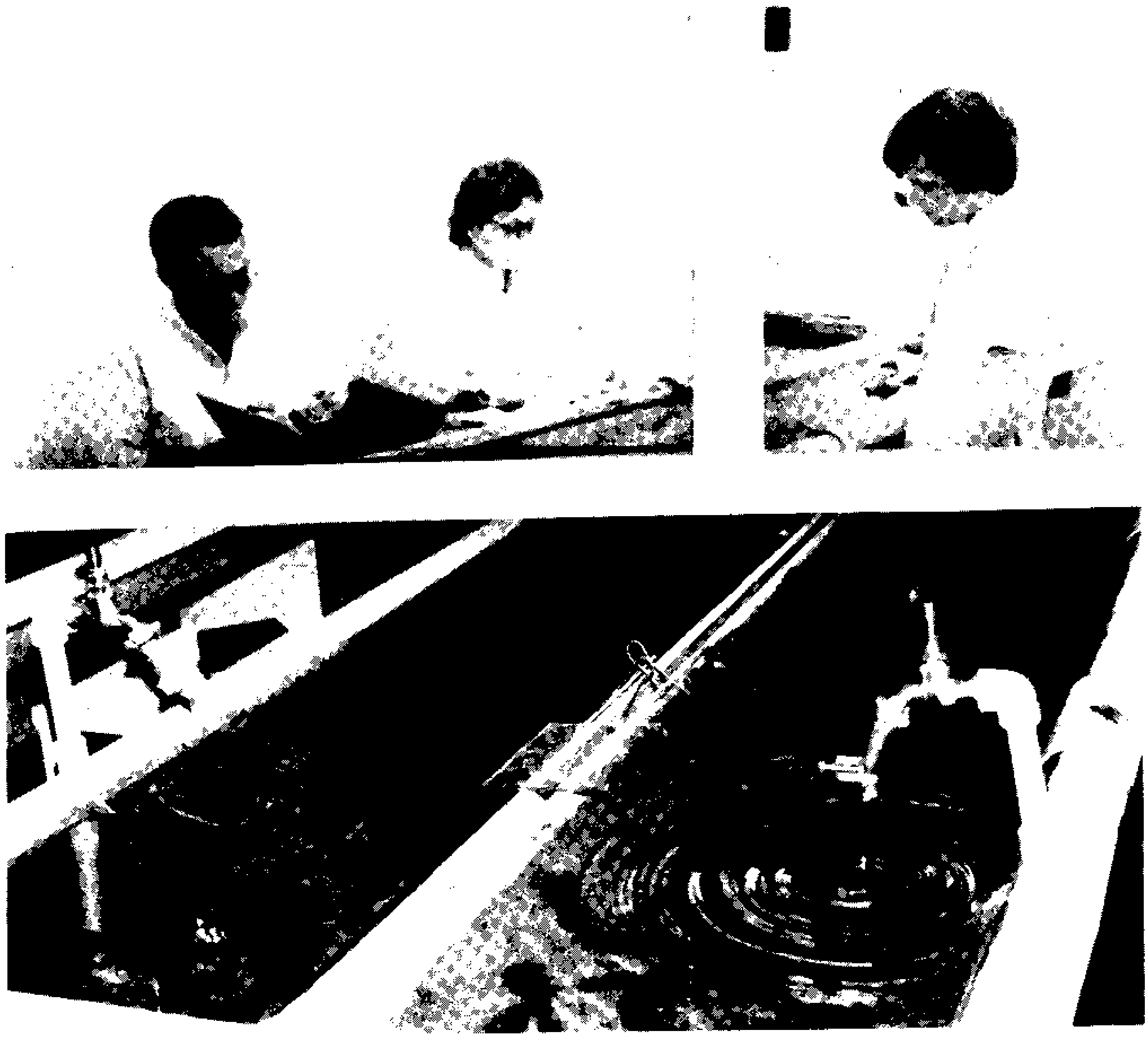

Fig. 4 – Some aspects of the work at the channels: a. snail counting and first inspection of the plants (one counting station is seen out of water); b. aspiration of excess organic matter; c. water feeding mechanism.

Luxuriant snail cultures are being maintained in the laboratory channels for about two and a half years and there are no reasons to suppose that this cannot be indefinitely extended.

The maintenance of *Biomphalaria* populations, under seminatural conditions, represents a technical advance in malacological work.

Controlled indoor environment techniques may also be utilized for different types of experiments in malacology.

It is believed that studies on snail population interactions may lead to the development of a methodology that could be used in the control of the snail intermediate host of the schistosomes.

RESUMO

Com a finalidade de realizar experimentos de laboratório sobre interações em populações de caramujos de água doce, salas foram especialmente preparadas para este fim no edifício principal do Centro de Pesquisas Aggeu Magalhães. Nesta publicação, a primeira de uma série sobre o assunto, é descrita, com detalhes, a metodologia geral do trabalho de laboratório. Utilizando canais de cimento foram estabelecidas condições semi-naturais sob as quais puderam ser estudadas as interações de populações de caramujos com um mínimo de interferência de variáveis ambientais. Técnicas que utilizam controle de ambiente interno, como as descritas no presente trabalho, podem ser também empregadas em muitos outros tipos de experiências em malacologia e constituem avanço substancial em trabalhos malacológicos.

ACKNOWLEDGMENTS

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