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BREEDING OF *Biomphalaria tenagophila* IN MASS SCALE

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SUMMARY

An efficient method for breeding *Biomphalaria tenagophila* (Taim lineage/RS) was developed over a 5-year-period (2005-2010). Special facilities were provided which consisted of four cement tanks (9.4 x 0.6 x 0.22 m), with their bottom covered with a layer of sterilized red earth and calcium carbonate. Standard measures were adopted, as follows: each tank should contain an average of 3000 specimens, and would be provided with a daily ration of 35,000 mg complemented with lettuce. A green-house effect heating system was developed which constituted of movable dark canvas covers, which allowed the temperature to be controlled between 20 - 24 °C. This system was essential, especially during the coldest months of the year. Approximately 27,000 specimens with a diameter of 12 mm or more were produced during a 14-month-period. The mortality rates of the newly-hatched and adult snails were 77% and 37%, respectively. The follow-up of the development system related to 310 specimens of *B. tenagophila* demonstrated that 70-day-old snails reached an average of 17.0 ± 0.9 mm diameter. The mortality rates and the development performance of *B. tenagophila* snails can be considered as highly satisfactory, when compared with other results in literature related to works carried out with different species of the *genus Biomphalaria*, under controlled laboratory conditions.

KEYWORDS: Mass scale breeding; *Biomphalaria*; *Biomphalaria tenagophila*; Resistant lineage; *Schistosoma mansoni.*

INTRODUCTION

The mass scale breeding of snails genus *Biomphalaria* under laboratory conditions has been described by several authors, and the great majority of published works were carried out using *Biomphalaria glabrata*, not only because it is the species with the highest susceptibility to *Schistosoma mansoni*, but also for ease of breeding and maintenance under laboratory conditions[7,14,23,26,28,30]. Specimens of *B. glabrata* infected under laboratory conditions can eliminate an average of 6,061 cercariae per snail[29]. The good reproductive performance of *B. glabrata* is also another favorable factor [14,15,16,21]. After the establishment of the evolutive cycle of *S. mansoni* under laboratory conditions, it was possible to conduct basic and applied researches on schistosomiasis, resulting in many thousands of scientific articles being published in specialized journals, as well as allowing the production of antigens from cercariae, schistosomules, worms, and *S. mansoni* eggs, which were and will be used in diagnostic methods and the development of vaccines. Some techniques related to the breeding of *Biomphalaria* in large scale that were already published refer to breeding under laboratory conditions, and the major fact analyzed was generally the type of food provided[8,23,24]. The doctoral thesis presented by FREITAS (1973)[14] represents the most complete work related to the growth rhythm of *B. glabrata*, and the standardization of the technique for large scale breeding. In the mentioned work, factors such as temperature, lighting, water quality, amount of food available, and the influence of the "crowding" effect were studied, aiming at improving the breeding techniques, and above all to evaluate the ideal conditions for the proper development of the snail. Although *B. tenagophila* and *B. straminea* are less susceptible to *S. mansoni* than *B.glabrata*, they have an important role in schistosomiasis transmission in South, Southeast, and Northeast regions in Brazil. The need for breeding albino *B. straminea* (resistant to *S. mansoni* infection), on a large scale, gave rise to an efficient technique described by BARBOSA & BARBOSA in 1995[2]. As far as *B. tenagophila* is concerned, up until now there has been no literary publication related to the standardization of a method for the large scale breeding of this species. The few works that do exist concerning the development of *B. tenagophila,* were conducted under laboratory conditions by KAWAZOE in 1976 and 1977[15,16]. Studies related to the embrionary development of *B. glabrata* and *B. tenagophila*, under laboratory conditions for a 1-year-period, demonstrated that there are no significant differences in the embrionary period duration between the two species[15]. The averages of embrionary period detected for *B. glabrata* and *B. straminea* were 7.6 and eight days, respectively. The same author observed that *B. glabrata* was more fertile than *B. tenagophila*[16].

The very few works that specifically target studies on the development rhythm, as well as on the breeding of *B. tenagophila* on a mass scale, constituted the major difficulty found by our research group for obtaining the production of thousands of *B. tenagophila* (Taim strain) specimens.

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This strain is absolutely resistant to *S. mansoni,* and has been the subject of various studies[1,5,6,13,17,18,19,20,25,27]. Among these studies, the model for the biological control of schistosomiasis transmission is highlighted. It consists of the transference of resistance from *B. tenagophila* to *S. mansoni* (Taim/RS) to the local susceptible lineage by means of crossbreeding, since the resistance gene has a dominant character[25]. Nevertheless, in order to test this model in field, numerous specimens of *B. tenagophila* Taim are required in areas where the transmission is maintained by *B. tenagophila*[11]. The municipality of Bananal, in the State of São Paulo, Brazil, was the area chosen for introduction, taking into account the comprehensive malacological studies and the evidence of the exclusive presence of *B. tenagophila* in the mentioned area[31].

The aim of the present work was to establish an effective method for breeding *B. tenagophila* (Taim lineage) specimens in large scale. This would be of the utmost importance to meet the great demand for specimens used in experiments performed under field and laboratory conditions, and may also serve as a basis for future studies with other species of the genus *Biomphalaria.*

METHODOLOGY

Breeding of B. tenagophila (Taim) in large scale

Location and assembly of the tanks: With the purpose of introducing *B. tenagophila* (Taim, RS) in endemic areas where *B. tenagophila* is the unique transmitter species, a large scale breeding program was launched at the "Chácara Santa Inês - Vila Bom Jardim" in Bananal, State of São Paulo. Throughout the period 2005-2010, the snails were successfully bred in a shed. From 2010 onwards, new facilities for mass scale breeding were designed, which were bigger and better adapted for the purpose of breeding *Biomphalaria* (Fig. 1 A e B). The new facilities consist of four large cement tanks, measuring 9.4 m long x 0.2 m deep x 0.6 m wide, with a capacity of 1240 liters. The bottom of each tank was covered with a layer of red earth, sterilized in an oven at 150 °C, plus 10% calcium carbonate. All tanks contain a PVC pipe in the center, which is 0.2 m high, and topped with screen functioning as a drain for the water change. The bottom of the tanks has a stopper, which may be removed, thus facilitating the flow of water during the periodic cleaning process. In order to prevent Culicidae females from performing oviposition in the tanks, as well as the escape of molluscs, the tanks were covered with a nylon netting fixed to four wooden slats measuring 3 m x 0.60 m, with two hinges at the upper side. The covers are opened using a movable system consisting of a nylon rope, which when pulled out with the aid of a pulley on the ceiling, raises the covers to a 90° angle, and it can remain fixed at this angle. This movable system was designed to facilitate management (Fig. 1C).

The temperature of the tanks is controlled by covers made of canvas (black on the outside and white inside) which are used in sheds designed for breeding chickens. These covers are not used during the hottest months, but in winter they are ringed down in order to protect the whole tank area. These canvas covers keep and concentrate the heat from the sun by green-house effect, which allows the water temperature of the tanks to be controlled between 20 to 24 °C; this is especially important during the coldest months. There are two types of lighting**:** natural lighting when the covers are raised, since the tanks get a direct light from the sun; and an artificial one using fluorescent lamps, mainly when the covers are ringed down during the winter period or at night, when necessary (Fig. 1 D).

Fig 1 - Breeding of *B. tenagophila* in mass scale at the "Chácara Santa Inês", Bananal/ SP. A: New facilities for breeding of *B. tenagophila* (Taim lineage) (inside view). **B:** Obs.: Breeding of *B. tenagophila* from Taim (outside view). (*)The canvas is black on the outside and white inside. **C:** New facilities for breeding *B. tenagophila* (Taim lineage) on a mass scale, showing the system for opening and raising the covers of the breeding tanks and showing protection for the tanks by means of black canvas for temperature maintenance through greenhouse effect. **D:** The former facilities for breeding *B. tenagophila* (Taim lineage), showing protection for the tanks by means of black canvas for temperature maintenance through greenhouse effect. **E:** Tank for breeding *B. tenagophila* (Taim lineage) showing the snails, the bottom covered with earth, and Styrofoam plates for catching the spawnings. **F:** Measurer used to assess the growth rhythm of *B. tenagophila* (Taim lineage).

The water supply for the tanks derives directly from a local source, with daily circulation, and is 7.0 pH, approximately. The tank faucets are covered with a nylon net in order to avoid the access of other organisms, such as leeches, other molluscs, insect larvae, etc., which could be brought with the water and cause serious problems to the snails´ breeding.

Breeding and Feeding: Specimens of *B. tenagophila* (Taim strain), resistant to *S. mansoni*, were collected at the Ecological Station of Taim, State of Rio Grande do Sul, in 1979. Since then the molluscs have been maintained in the mollusc room, at the Laboratory of Schistosomiasis, Department of Parasitology, ICB/UFMG. The colony of snails used for mass scale breeding derived from the specimens maintained in that

Laboratory. In order to launch breeding in 2005, 1,200 snails were distributed into a single cement tank, and Styrofoam plates were placed on the water surface with the purpose of collecting spawnings (Fig. 1 E). The plates full of spawnings were then transferred to a new "nursery" tank, where eclosion occurs and the newly born specimens are reared. When they reached a diameter of 4-5 mm, the snails were transferred from the "nursery" to the appropriate breeding tanks. The diet for the snails was similar to that used in the mollusc room at the Laboratory of Schistosomiasis, ICB/UFMG. This diet consists of fresh lettuce leaves and ration. The ration for snails was standardized by FREITAS[14], and it is composed of 1 kg alfafa plus 1 kg ration for mice, 500 g wheat germ, 300 g calcium carbonate, 300 mg vitamin E and 100 g powered milk. It was established that each tank would shelter 3000 specimens on average, and would be provided with a daily ration of 35,000 mg, whereas the "nursery" tanks would receive 25,000 mg of the same ration one week before the eclosion, in order to form a layer of algae and fungi on the bottom and the walls of the tanks. This is the main nutritional source for the newly-hatched snails[14]. The aim being to strengthen the snails´ shells, 20 g calcium carbonate was added to each tank every 15 days. The above mentioned conditions were also maintained at the beginning of the current breeding carried out at the new facilities.

Mortality rate: Due to the difficulty of estimating the mortality rate of the emerging snails throughout this experiment, since they are very small, this parameter was evaluated as follows: 415 spawnings containing a total of 10,249 eggs of *B. tenagophila (Taim lineage)* were transferred to the "nursery" tank. These eggs were counted under a stereomicroscope. Thirty-days later, the number of living snails was evaluated, thus the survival rate could be estimated and after that the mortality rate could also be obtained. The number of dead snails in the breeding tanks was weekly recorded, for a 14-month-period.

Development rhythm of B. tenagophila (Taim lineage): In order to evaluate the development rhythm of *B. tenagophila* Taim, 310 specimens with a diameter of 5 mm and approximately 20 days of age, were transferred to a determinate part of the breeding tank. The area occupied was 2 m long x 0.2 m deep x 0.6 wide. The snails were kept under the same conditions previously described, except for the amount of food daily offered (3,500 mg ration). The snails were measured on days 20, 30, 40, 50, 60 and 70 by means of a measuring device consisting of a plastic net with a checkered bottom on which diameter circles of between 2 to 36 mm were drawn (Fig. 1 F).

RESULTS

1. The culture of *B. tenagophila* (Taim) in mass scale at the "Chácara Santa Inês" was set in full operation six years ago. The data showed in this work refer to the new facilities, for the period October 2010 to November 2011. Approximately, 27,000 specimens were produced in the breeding tanks for a 14-month-period. One thousand and nine hundred molluscs are produced per month, with a diameter of 12 mm or more. Some aspects such as water quality, temperature control (even in winter), as well as space availability were of great importance for the production of the resistant lineage on a mass scale throughout the year.

2. The mortality rate among adult specimens did not surpass 37% during the studied period, however these values were higher among the newly-hatched snails. From the 10,294 eggs of *B. tenagophila* Taim placed into the "nursery" tank, only 2,345 specimens were able to survive up to 30 days post-eclosion, and the mortality rate of this group was 77%. On the other hand, the surviving specimens transferred to the breeding tanks grew rapidly and, consequently, after sexual maturation, showed an intense reproduction in the tanks. It was observed that in the "nursery" tank a high number of embrios hatched around the 6th day.

3. Due to inumerous difficulties involved in monitoring the growth rhythm of all *B. tenagophila* Taim specimens, starting from their eclosion up to the adult phase, it was established that such procedure should be performed with 310 specimens presenting 5 mm diameter, with an age grade of ± 20 days. As can be seen in Fig. 2, the growth rhythm of the molluscs evaluated up to 70 days was very uniform. At the beginning of the experiment, the snails measuring 5 mm diameter reached 8.0 ± 1.3 mm diameter one month after eclosion. These snails attained 3 mm diameter in 10 days. After completing 40 days of age, the molluscs were again evaluated, and the mean obtained for their shells was 10.8 ± 2.6 mm diameter. The value obtained for the 50-day-old snails was 13.0 ± 3.1 mm diameter. Specimens with 60 and 70 days of age presented shells measuring 15.0 ± 1.4 mm and 17.0 ± 0.9 mm diameters, respectively. As shown in Fig. 2, the mortality rates were 2.6% and 2.0% for the periods of 20-30 and 30-40 days, respectively. It could be observed that the higher mortality rate (25.3%) occurred between 40-50 days, and that no deaths occurred in the breeding tank after a 50-60-day-period. The mortality rate for the period of 60 to 70 days was 6.8%. After 70 days the accumulated mortality rate was estimated as 33.5%.

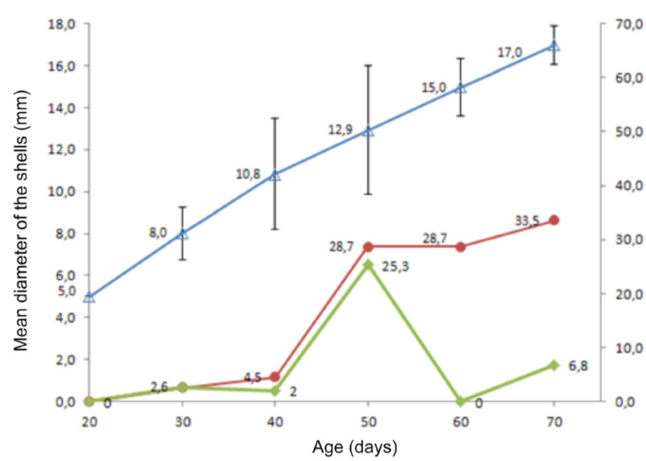

Fig. 2 - Graphic showing the growth and mortality rates of *B. tenagophila* (Taim lineage) in the breeding tanks, Bananal/SP. △ Mean growth, ● Accumulated mortality rate, ◆ Mortality rate per period.

DISCUSSION

Breeding of *Biomphalaria tenagophila* (Taim lineage) was successfully maintained under laboratory conditions for more than 27 years. The demand for specimens in various experiments which were performed in recent years, required thousands of individuals in experiments carried out both in field and laboratory. This fact led to the need to develop a standard technique, ideal for the breeding of that strain on a mass scale. Although some efficient techniques for breeding *B. glabrata* under laboratory conditions have already been described[14,23], our technique was performed under a semi-open environment, using *B.*

tenagophila strain, and we selected some mentioned procedures by those authors as a basis for our study. The facilities described in the present work were very different from those used under laboratory conditions. The *B. tenagophila* snails were reared in big cement tanks, with the tank bottom covered with a mixture of earth and calcium carbonate. This system was proved to be highly efficient and of low cost for the production of snails on a large scale.

Temperature control is a crucial point for the good development of aquatic snails[14]. FREITAS[14] reports that during the standardization of the breeding technique *B. glabrata* the glass aquaria were kept in a room with a constant temperature of around 25 °C, and in winter the heaters were activated to avoid a sudden drop of the temperature. At the beginning, this temperature control system was practically impossible in the shed due to its large extension, as well as due to the high consumption of electric energy required by the heaters used. Therefore, the use of black canvas covers was an alternative developed in order to control the temperature (between 20 -24 °C), mainly in winter. The use of this system allowed stabilization of the water temperature and also resulted in the good development and reproductive performance of the snails, even in winter, without electric energy consumption.

In the present study, it was established that each breeding tank would receive 3000 snails on average, as a standard procedure. In this manner, the super population effect was avoided, the so called “crowding effect”. This occurs when a determined population reaches the critical number of individuals, that is, a higher density of molluscs for the same water quantity. This may lead to a decrease in productivity, as well as an increase in mortality rates[2,4,9]. COELHO *et al.*[10] verified, by means of the caption technique of radioisotope (^{59}Fe), that an increase in the populational density corresponds to a decrease in the incorporation capacity of ^{59}Fe. The same authors also observed a decrease in the snails´ growth, as well as an increase in the mortality rate of the snails in the aquaria with high population densities.

As far as the breeding techniques are concerned, an adequate supply of food is one of the most important factors for regulation of the snails´growth rates. At the beginning of our work, when the population reached approximately 3000 snails, these snails received 25,000 mg of ration plus a supplement of lettuce *ad libitum* every two weeks, that is, in sufficient quantity to be consumed on the day, thus avoiding brewing water and, consequently, the snails´ death. Nevertheless, it was verified that the amount of food offered was not sufficient, since the snails measuring 5 mm diameter showed a very slow growth rhythm. After this fact, each breeding tank was supplied with 35,000 mg ration every day, plus fresh lettuce as a supplement, and it was consequently observed that the snails attained a marked development rhythm. However, the diameter of the mollusc shells was not recorded on that occasion. The same behavior was noted in studies performed with *B. glabrata*[14]. The increase from 1.4 mg/snail/day to 11.7 mg/day in the experiments resulted in a sudden high growth rate. The snails measuring 5.6 mm ± 1.8 mm diameter, started reaching 11.3 mm diameter within one month of age. The emerging snails showed a peculiar feature, after this development stage they did not eat ration, but they fed on fungi and algae formed at the bottom of the aquaria. Previous studies demonstrated that by using this method it is possible to obtain more than 90% of surviving snails in the “nursery” glass aquaria[14]. Although when this procedure was also performed by our research group, we detected a mortality rate of 70% among the emerging snails in the so called “nursery” tank. Nevertheless, these rates are in accordance with the surviving rate of different aquatic organisms artificially reared, such as fingerlings in pisciculture, and other molluscs in commercial breeding, such as *escargots* and oysters. It is important to remark that our system is considered semi-natural, different from the laboratory environment, with well controlled variables. It is well known that in nature the newly-born organisms from different animals´ orders and species present a very high mortality rate under natural conditions, with a very low rate of survivors up to the adult phase. The large number of animals produced acts as a compensatory factor for mortality that allows the maintenance of the species in the natural environment, this occurs mainly with invertebrates. Various authors mention that one of the most critical points concerning the breeding of molluscs is the development of newly-hatched snails, since the highest mortality rate occurs at this stage[3,12,22,30].

In relation to the growth rhythm of *B. tenagophila,* which is poorly known, it was verified that *B. tenagophila* (Taim lineage) presented a good growth rhythm in the breeding tanks since after 70 days the specimens reached 16.98 mm diameter on average. Although these data were obtained with a small population, consisting of 310 individuals, the values obtained were very similar to those observed in the other tanks. Studies conducted with *B. glabrata* mention that 30-day-old individuals are able to reach a diameter of 17.0 mm on average, increasing to 23.0 mm diameter after 60 days[14]. The values related to *B. glabrata,* as mentioned by the same author, were higher than other ones reported by various researchers, and all the mentioned works by them were carried out under laboratory conditions, with very well controlled variables[8,24].

The present study presents an efficient methodology for breeding *Biomphalaria* on a mass scale with economical facilities, low cost of production and ease of maintenance.

RESUMO

Criação em larga escala de *Biomphalaria tenagophila*

Foi desenvolvido um método eficiente de criação em larga escala de *Biomphalaria tenagophila* (linhagem Taim/RS) durante o período de 2005-2010. Foi concebida uma instalação que consiste de quatro tanques de alvenaria (9,4 x 0,6 x 0,22) com fundos recobertos por uma mistura constituída de terra vermelha esterilizada e carbonato de cálcio. Foi padronizado que cada tanque de criação conteria em média 3.000 exemplares e receberia diariamente 35.000 mg de ração e alface como complemento. O desenvolvimento de um sistema de aquecimento por efeito estufa constituído de lonas escuras móveis permitiu controlar a temperatura entre 20 a 24 °C, sistema essencial principalmente nos meses mais frios. Durante o período de 14 meses foram produzidos aproximadamente 27.000 exemplares com diâmetros superiores a 12 mm. As taxas de mortalidade dos caramujos recém-eclodidos e adultos foram de 77% e 37%, respectivamente. O acompanhamento do ritmo de crescimento de 310 *B. tenagophila* demonstrou que caramujos com 70 dias de idade alcançaram em média 17,0 ± 0,9 mm de diâmetro. As taxas de mortalidade e o desempenho de crescimento de caramujos do gênero *B. tenagophila* podem ser considerados altamente satisfatórios, comparando-se com os resultados da literatura realizados com espécies do gênero *Biomphalaria* em condições controladas de laboratório.

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AUTHOR CONTRIBUTIONS

Florence Mara Rosa participated in the preparation of the project for the new facilities related to the mollusc room; helped with the formulation and control of feeding the molluscs; participated in all steps to establish the basic procedures for the mollusc breeding; participated in the experimental design to assess the growth and mortality rates of the snails, and participated in the writing of this article as well.

Daisymara P. Almeida Marques participated actively in the assembly and planning of the new breeding facilities; participated in the discussion and execution of the experiments and participated in the writing of this article.

Engels Maciel is the owner of the realty where the mollusc room was installed. As a retired Engineer in the research area of the "Companhia Vale do Rio Doce"; he had an enthusiastic approach and a fundamental role in the assembly of the breeding facilities. His suggestions, based on his experience of Engineering and previous researches constituted the central focus of the improved model.

Josiane Maria Couto, student of Biology, participated actively in the process of assembly and maintenance of the breeding tanks, and contributed with important suggestions for the improvement of the methodology.

Deborah A. Negrão-Correa, since the beginning of the breeding process she has given valuable suggestions for improving the breeding process, using her extensive experience in maintenance of *Biomphalaria* in laboratory; she provided us with the specimens used in the beginning of this breeding process; and also participated in the planning of the experiments and in the writing of this article.

Horácio M. Santana Teles participated in the assembly of the facilities related to the tanks and the mollusc room. His high level of experience as Malacologist at SUCEN was very important for the improvement of the breeding system of *Biomphalaria;* he also participated in the preparation of the experiments, as well as in the discussion of the results.

João Batista dos Santos worked intensively throughout all stages of the assembly of the breeding system. His dedication was of fundamental importance for the success of the model designed; he also presented valuable suggestions for improvement of the management and efficiency of the breeding process.

Paulo Marcos Zech Coelho is the Coordinator of this project, and participated in all stages of the model

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