Rearing of *Lymnaea columella* (Say, 1817), Intermediate Host of *Fasciola hepatica* (Linnaeus, 1758)

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The intermediate host of Fasciola hepatica, Lymnaea columella, *collected in Belo Horizonte, Minas Gerais, Brazil, was reared in our laboratory. The aim of the current study was to standardize a rearing and maintenance technique. Two kinds of diet were tested: fresh lettuce (A) and rodent ration + 10% $CaCO_3$ plus fresh lettuce (B). The age for the beginning of oviposition ranged from 27 to 57 days. Ten days after oviposition at 24.7°C, 100% eclosion occurred. The complete life cycle varied from 37 to 67 days. The average numbers of eggs per egg mass were 26.3 and 31.1 with diets (A) and (B), respectively. The lettuce and ration fed snails presented a increased growth although the difference was not statistically significant ($p > 0.05$). The mortality rate varied from 40 to 64% after 90 days. The maximum longevity was 183 days, 21.5 mm length and 11 mm wide. The methodology to mass breed and maintain these snails was found to be suitable in the laboratory*

Key words: fasciolosis - *Lymnaea columella* - intermediate host - Minas Gerais - Brazil - rearing techniques

The geographic distribution of Lymnaeidae is very wide in Brazil (Paraense 1982a,b, 1983, 1986). In Minas Gerais, *Lymnaea columella* specimens from Sete Lagoas, Santa Rita do Sapucaí, Viçosa and Belo Horizonte municipalities were found to be susceptible to experimental infection with *Fasciola hepatica* miracidia (Dacal et al. 1988). Recently, Silva et al. (1995) reported the first natural infection of *L. columella* with *F. hepatica* in Itajubá, southern of Minas Gerais. In a malacology survey performed in 13 municipalities in the Belo Horizonte microregion, *L. columella* was found in eight: Pedro Leopoldo, Lagoa Santa, Santa Luzia, Vespasiano, Ribeirão das Neves, Belo Horizonte, Nova Lima and Betim (Souza et al. 1998).

This study initiated the rearing of *L. columella*, collected in Belo Horizonte. The aims of the study were to standardize a breeding and maintenance technique for these snails collecting data on growth, oviposition, number of eggs per egg mass, egg incubation period and longevity. In the future, these snails will be submitted to experimental infection with *F. hepatica* in order to obtain the eggs, cercariae, metacercariae and the adult worms to study vaccine production.

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MATERIALS AND METHODS

Snails used in this study were collected from a breeding colony at Pontifícia Universidade Católica campus in Belo Horizonte, MG, in March 1998. After identification by Dr WL Paraense from the Malacology Department of Instituto Oswaldo Cruz, Rio de Janeiro the snails were divided in two groups of 25 and placed in two 60 x 30 x 28 cm glass aquaria. Each aquarium containing approximately 10 l of dechlorinated water was provided with a water stream during 8 h per day. As substrate, sterilized soil + 10% calcium carbonate ($CaCO_3$) was added to the water. The aquaria were maintained on metal shelves with natural light exposure. Two samples of water containing algae and the aquatic plant (*Lemna* sp.) were added to the aquaria. The water temperature and pH were monitored daily. To collect the eggs, uncolored plastic sheets were put into the two aquaria (Olivier 1960). To control predators such as oligochaetes worms and ostracods, which could have come from the field with the snails, acetic acid was used to wash the contaminated aquaria.

Experiments - Two experiments testing two diets were performed. The snails in aquarium A were fed fresh lettuce and in B, fresh lettuce plus rodent powered ration (Purina®) + 10% $CaCO_3$; the latter diet has already been used to feed *Biomphalaria* in our laboratory (Souza et al. 1985, 1987). The paste ration was placed on the lettuce leaves. In the first experiment, 100 newly hatched snails were used, 50 in each aquarium. In experiment two, 343 specimens were used in A aquarium and 401 in B.

Snail development was evaluated by measurement and counting after 30, 60, 90, 120 and 150 days post eclosion. Any dead specimens were removed. To observe longevity in the laboratory, in experiment two, 20 specimens which were 60 days old, from each aquarium were marked with enamel.

Statistical analysis: the average reared snail lengths were compared using Student t test ($p \geq 0.05$).

RESULTS

Egg eclosion occurred 10 days after oviposition, at 24.7°C and the eclosion rate was 100% in both experiments. The age for the first oviposition ranged from 27 days in the first experiment and 57 days in the second experiment. The complete life cycle ranged from 37 to 67 days. The average egg snails per egg mass was 26.3 and 31.1 for aquaria A and B, respectively.

Data on snails growth and mortality in the two experiments, and the average water temperatures are shown in Tables I and II. The snails which were fed with lettuce plus ration developed faster than those in the other group, although the difference was not statistically significant ($p > 0.05$). After 90 days 60 to 64% of mortality was observed among the snails of the first experiment and 40.89 to 41.69% among the snails of the second experiment (Tables I, II). Water pH varied from 6.7 to 6.9 and the temperature from 24.7 to 27.6°C.

From March 1998 to March 1999 approximately eight *L. columella* generations and 3,000 additional specimens were obtained in six aquaria in our laboratory. During the hottest months, December 1998, January and February 1999, the mortality of adult snails was higher.

TABLE I

Growth and mortality of *Lymnaea columella* reared in the laboratory, fed with lettuce (A) and lettuce plus rodent ration + 10% $CaCO_3$ (B), in the experiment 1

Aquaria	Number of snails	Age (day)	Average length (mm)	Average width (mm)	Mortality rate (%)	Average temperature (°C)
A	50	1	1.11	0.68	0.0	25.1
B	50	1	1.11	0.68	0.0	25.2
A	50	30	4.78	2.21	0.0	25.1
B	50	30	4.64	2.30	0.0	25.2
A	41	60	14.21	7.09	18.0	24.7
B	48	60	15.39	7.60	4.0	24.7
A	18	90	18.17	8.55	64.0	25.3
B	20	90	19.62	9.04	60.0	25.4

The beginning of the eggs output occurred at 27 days after eclosion.

TABLE II

Growth and mortality of *Lymnaea columella* reared in the laboratory, fed with lettuce (A) and lettuce plus rodent ration + 10% $CaCO_3$ (B), in the experiment 2

Aquaria	Number of snails	Age (day)	Average length (mm)	Average width (mm)	Mortality rate (%)	Average temperature (°C)
A	343	1	1.11	0.68	0.0	24.7
B	401	1	1.11	0.68	0.0	24.7
A	343	30	2.41	1.16	0.0	24.7
B	401	30	3.03	1.44	0.0	24.7
A	335	60	6.85	3.45	2.33	27.6
B	395	60	7.59	3.87	1.49	27.4
A	200	90	12.21	5.37	41.69	25.0
B	237	90	14.42	7.84	40.89	25.1
A	147	120	14.71	7.63	57.14	26.1
B	152	120	18.25	8.76	62.09	26.0
A	32	150	17.01	8.62	90.6	25.0
B	102	150	18.50	9.62	74.5	24.9

The beginning of the eggs output occurred at 57 days after eclosion.

The greatest *L. columella* longevity period recorded in these experiments was 183 days. The length was 21.5 mm and the width 11 mm.

DISCUSSION

Several authors have developed *Lymnaea* snail rearing techniques in the laboratory (Noland & Reichel 1943, Madsen & Monrad 1981, Sanchez et al. 1995). However, these techniques are highly variable and special techniques must be used when rearing species such as *Fossaria cubensis* and *L. columella*, the *F. hepatica* hosts in Cuba and Brazil, respectively, which are amphibious snails.

Snail rearing techniques, maintenance of the aquaria and diet are extremely variable. Preferentially, small recipients including Petri dish and crystallizing recipients are used. Large water tanks are also used, mainly to grow algae to feed the snails.

In the present study, the methodology used to rear *L. columella* in the laboratory was the same as that used for *B. glabrata* mass growing (Souza et al. 1985, 1987), with satisfactory results (Tables I, II). Initially, in addition to lettuce and powered ration, algae and the aquatic plant (*Lemna* sp.) were introduced into the aquaria, but because of the great voracity of the snails the feeding was simplified to just lettuce and lettuce plus ration, + 10% $CaCO_3$.

The effects of snail crowding in population were observed in one of the experiments as reported by Gazzinelli et al. (1970), in *B. glabrata*. In the former, the 50 snails developed faster than those in the latter experiment in which 343 and 401 specimens were growing together (Tables I, II).

The age of the snails for the first oviposition varied from 27 to 57 days, being higher than that reported for *F. cubensis* which ranged from 18 to 20 days after eclosion (Sanchez et al. 1995). The snail mortality rates ranged from 40 to 64% at 90 days old (Tables I, II) indicating that infections with *F. hepatica* should be performed at around 30 days old (2-5 mm length). The largest size registered after 183 days for *L. columella*, from Belo Horizonte, and reared in our laboratory was 21.5 mm in length and 11 mm in width; these measurements exceed those reported for specimens from different regions of the country (Paraense 1983, 1986).

The methodology used to rear and maintain these snails was found to be suitable in the laboratory. The preferred diet to rear *Lymnaea* is lettuce plus powered ration + 10% $CaCO_3$ the same as for *Biomphalaria*.

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