

# Infection by *Schistosoma mansoni* Sambon 1907 in the First Four Months of Life of *Biomphalaria straminea* (Dunker, 1848) in Brazil

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*Compatibility between Schistosoma mansoni and Biomphalaria straminea when exposed to the parasite on the first four months of age was assessed for five parasitological aspects: indices of infection and mortality, duration of precercarial and cercarial periods, and rate of cercarial emission. Infections were made on molluscs from laboratory colonies, at the following ages: 8, 13, 18, 21, 53, 83 and 114 days. Two B. straminea colonies were used (Camorim, PE and Picos, PI), and one B. glabrata colony (Ressaca, MG) was used as control. The main results are as follows: (I) infection was significantly associated with mollusc age, being proportionally higher in sexually immature than in mature molluscs for the three colonies; (II) for B. straminea from Camorim, mortality did not differ significantly between infected and non-infected snails; for B. straminea from Picos significantly more deaths occurred among infected than among non-infected snails, while the opposite was observed for B. glabrata from Ressaca; (III) for the three colonies, the precercarial period was significantly shorter for immature molluscs than for mature ones; (IV) the duration of the cercarial period was extremely variable for the three colonies; (V) sexual maturity did not influence cercarial emission for the three colonies.*

Key words: *Schistosoma mansoni* - *Biomphalaria straminea* - compatibility - age - Brazil

One of the factors influencing the intermediate host susceptibility to *Schistosoma* spp. is the mollusc developmental stage. Archibald and Marshall (1932) were the first ones to observe the decrease of the susceptibility with the mollusc age in the combination *Schistosoma haematobium* and several species of *Bulinus*. Other authors (Moore et al. 1953, Chu et al. 1966, Raymond & Probert 1992) confirmed that observation and pointed out other parasitological aspects: survival, duration of the precercarial period and cercarial emission.

However, the influence of age in the combination *Schistosoma mansoni*-*Biomphalaria* is not completely defined. Newton (1953), using *Biomphalaria glabrata*, proved that molluscs at the age of 1 to 9 days are highly susceptible and the infection rate on older snails is significantly lower. Richards (1970) identified four categories of susceptibility of *B. glabrata* to *S. mansoni*: (1) non susceptible at any age; (2) juvenile susceptible/

adult non susceptible; (3) susceptible at any age; and (4) juvenile susceptible/adult variable. According to Michelson and Dubois (1978) refractory populations show different degrees of susceptibility when submitted to experimental infection in newly-hatched molluscs. According to Niemann and Lewis (1990), *B. glabrata* susceptibility and *S. mansoni* cercarial production are strongly influenced by the size of the host and not by its age. Chernin and Antolics (1975) and Richards (1973), using *Biomphalaria straminea* colonies, emphasized the importance of young molluscs in the *S. mansoni* transmission dynamics. More recently, Fernandez (1997), using *B. glabrata*, *Biomphalaria tenagophila* and *B. straminea* exposed to *S. mansoni* sympatric miracidia at the age of 1, 2 and 3 months, observed a decline in the susceptibility of *B. glabrata* and an increase in *Biomphalaria tenagophila*; for *B. straminea* no significant differences on the infection index were found.

In order to bring subsidies to clarify the bearing of immature molluscs on the dynamics of *S. mansoni* transmission, this study has investigated comparatively the compatibility between *B. straminea* and *S. mansoni* when exposed to the parasite on the first four months of age, including immature and mature stages. Five parasitological aspects were assessed: indices of infection and

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mortality, duration of precercarial and cercarial periods, and rate of cercarial emission.

### MATERIALS AND METHODS

*Snails populations* - Two *B. straminea* colonies were used (Camorim - São Lourenço da Mata, State of Pernambuco and Picos, State of Piauí), and one *B. glabrata* colony (Ressaca - Belo Horizonte, State of Minas Gerais) was used as control. The used snails were from colonies previously established in the Department of Malacology, Instituto Oswaldo Cruz. The colony from Camorim had been kept for approximately four years, while the others, for more than 13 years. The procedures for breeding the test specimens were described by Fernandez (1997).

*Experimental infection* - When the snails were 8, 13, 18, 21, 53, 83, and 114 days old, they were individually measured and exposed to five *S. mansoni* sympatric miracidia of the EC strain (*B. straminea* from Picos), of the CM strain (*B. straminea* from Camorim) and of the BH2 strain (*B. glabrata*). The EC and BH2 *S. mansoni* strains were isolated as described by Fernandez (1997). The CM *S. mansoni* strain was isolated on August 13, 1993 from feces of CM Braga, 4 years, born and grown up in Paud' alho - Engenho Pitangueira, PE, about 40 km from São Lourenço da Mata. There were 1,691 specimens of *B. straminea* used from Camorim, 1,972 specimens of *B. straminea* from Picos, and 1,735 specimens of *B. glabrata* from Ressaca. The procedures for collecting feces of infected mice and for later exposure of snails to miracidia were those described by Paraense and Corrêa (1989). The onset of egg laying was carefully observed on each aquarium to distinguish the molluscs exposed before the first oviposition (immature) from those exposed after having reached sexual maturity (mature). The aquaria were kept at a room temperature of 24-26°C throughout the experiment.

*Parasitological aspects* - Snails were observed daily and, if any specimen happened to die, it was fixed in Railliet-Henry's fluid and examined for developing stages of the schistosome. This procedure provided the mortality index (proportion of exposed molluscs that died during the precercarial period) on the three colonies. To characterize the duration of precercarial period and the infection index, the screening techniques used to detect the positive snails were those described by Paraense and Corrêa (1989); the specimens that survived for 70 days after exposure without shedding cercariae, were fixed, dissected and examined. From each age group in the three analyzed colonies, specimens that had emitted cercariae were separated at random to characterize the duration of the cercarial

period and the rate of cercarial emission. Each infected snail was kept separately in a small aquarium until death, and to estimate the cercarial emission the procedures described by Paraense and Corrêa (1989) were followed, except for the days of exposure (Mondays, Wednesdays and Fridays in the first month, and only Wednesdays in the following months). To confirm the parasitological cure, the mollusc was anesthetized, dissected and examined.

*Statistical analysis* - The statistical analysis were carried out using SYSTAT 7 (SPSS 1997). In order to ascertain whether there were significant differences, the data were analyzed through Contingency Tables (comparisons of infection and mortality indices related to age of exposure and stage of sexual development), Wilcoxon's test (infection and mortality indices), Analysis of Variance and Turkey's HSD Multiple Comparison test (duration of the precercarial and cercarial periods and cercarial emission), and Pearson's coefficient (correlation between the shell diameters and parasitological aspects).  $P < 0.05$  was considered significant.

### RESULTS

Results about infection and mortality of the *B. straminea* and *B. glabrata* colonies submitted to *S. mansoni* sympatric strains, as well as the diameter of molluscs when exposed to miracidia are in Table I. For the *B. straminea* colony from Camorim, 217 (12.8%) became infected, including 11 (5.1%) that died carrying sporocysts in the body tissues. From the 1,474 molluscs that remained negative, 54 specimens died (3.7%). From the 1,972 exposed molluscs of *B. straminea* from Picos, 114 (5.8%) were infected; among them, 9 (7.9%) died in the precercarial period. From the 1,858 molluscs that remained negative, 16 (0.9%) died in that same period. For the *B. glabrata* colony from Ressaca, 1,179 (68%) were infected; among them, 11 (0.9%) died in the precercarial period. From the 556 molluscs that remained negative, 25 (4.5%) died. Grouping the infection and mortality data by sexual maturation of molluscs when exposed to *S. mansoni*, indices were obtained for immature and mature stages, respectively, as follow: *B. straminea* from Camorim, 17.9 and 4.3% of infection and 0.9 and 8.8% of mortality; *B. straminea* from Picos, 7.7 and 3.2% of infection and 0.8 and 1.9% of mortality; *B. glabrata* from Ressaca, 83.6 and 48.2% of infection and 1.2 and 3.1% of mortality.

The duration of the precercarial period in the *B. straminea* colony from Camorim was  $34.8 \pm 8.62$  days (mean and standard deviation), varying from  $28.6 \pm 7.33$  days, on the 16 specimens exposed at the age of 21 days, to  $55 \pm 0$  days, on the two exposed at the age of 53 days. The duration of the precercarial period in the other ages was as fol-

lows: 8 days old,  $33.7 \pm 7.79$  days; 13 days old,  $34.3 \pm 6.85$  days; 18 days old,  $36.6 \pm 9.54$  days; 83 days old,  $52.5 \pm 9.81$  days; 114 days old,  $40.8 \pm 9.07$  days. For *B. straminea* from Picos, the precercarial period lasted  $31.9 \pm 5.42$  days, varying from  $27.9 \pm 3.41$  days, on the 19 specimens exposed at the age of 21 days, to  $44 \pm 0$  days, on the only mollusc that eliminated cercariae from the ones exposed at 83 days. The duration of the precercarial period in the other ages was: 8 days old,  $31.7 \pm 3.94$  days; 18 days old,  $32.3 \pm 5.19$  days; 21 days old,  $27.9 \pm 3.41$  days; 53 days old,  $35.3 \pm 6.37$  days; 114 days old,

$32.6 \pm 3.42$  days. For *B. glabrata*, this period lasted  $30.3 \pm 6.11$  days, varying from  $25.4 \pm 0.52$  days, on the 10 exposed at the age of 21 days, to  $33.9 \pm 5.41$  days, on the 13 exposed at 83 days. The duration of the precercarial period in the other ages was: 8 days old,  $28 \pm 4.13$  days; 13 days old,  $31.1 \pm 3.07$  days; 18 days old,  $29.8 \pm 8.23$  days; 53 days old,  $31.3 \pm 9.07$  days; 114 days old,  $32.5 \pm 5.03$  days. According to the sexual maturation of molluscs the following data were obtained for immature and mature stages, respectively:  $33.8 \pm 7.75$  days and  $45 \pm 10.37$  days on the specimens from Camorim;  $31.3 \pm$

TABLE I  
Infection and mortality of *Biomphalaria straminea* and *Biomphalaria glabrata* exposed at the ages of 8, 13, 18, 21, 53, 83 or 114 days to sympatric *Schistosoma mansoni*

Colonies of molluscs/ Strains of <i>S. mansoni</i>	Sexual stage of snails	Age of snails (days)	Number of snails exposed	Shell diameter (mm) (mean±SE)	% of snails infected	% of dead snails		
						+	-	total
<i>B. straminea</i> from Camorim (São Lourenço da Mata - PE)/ CM (Pitangueiras - PE)	Immature	8	237	$0.7 \pm 0.19$	30.38	1.39	0.61	0.84
		13	326	$1.3 \pm 0.35$	24.23	2.53	1.21	1.53
		18	281	$2.1 \pm 0.44$	7.83	0.0	0.39	0.36
		21	219	$3.2 \pm 0.41$	7.76	0.0	0.99	0.91
	Mature	53	246	$5.7 \pm 0.10$	1.22	0.0	0.82	0.81
		83	202	$7.3 \pm 0.43$	2.97	33.33	9.69	10.40
		114	180	$8.0 \pm 0.49$	10.00	33.33	16.05	17.78
<i>B. straminea</i> from Picos (Picos - PI)/ EC (Picos - PI)	Immature	8	334	$0.6 \pm 0.20$	8.68	6.90	0.33	0.90
		13	299	$1.2 \pm 0.27$	7.36	0.0	0.72	0.67
		18	297	$2.0 \pm 0.39$	5.72	5.88	0.36	0.67
		21	217	$3.1 \pm 0.31$	9.22	5.00	0.51	0.92
	Mature	53	234	$6.4 \pm 0.98$	3.42	12.50	0.44	0.85
		83	319	$8.0 \pm 1.24$	0.94	66.67	0.63	1.25
		114	272	$8.9 \pm 0.53$	5.51	13.33	3.11	3.68
<i>B. glabrata</i> from Ressaca (Belo Horizonte - MG)/ BH2 (Belo Horizonte - MG)	Immature	8	215	$1.0 \pm 0.0$	58.60	2.38	2.25	2.33
		13	279	$1.3 \pm 0.11$	92.47	0.38	4.76	0.72
		18	234	$2.1 \pm 0.22$	93.59	0.46	26.67	2.14
		21	240	$3.5 \pm 0.54$	85.83	0.0	0.0	0.0
	Mature	53	237	$8.8 \pm 0.66$	62.45	0.0	4.49	1.69
		83	281	$11.7 \pm 1.27$	39.86	0.89	4.14	2.85
		114	249	$12.8 \pm 0.64$	44.18	4.55	5.04	4.82

+: developing sporocysts; -: negative

5.38 days and  $34.1 \pm 5.17$  days, Picos;  $29 \pm 5.49$  days and  $32.6 \pm 6.58$  days, Ressaca.

As shown in Table II, the duration of the cercarial period was extremely variable for the three colonies. From the 143 Camorim snails observed, 7 (4.9%) were anesthetized and fixed since they have stopped cercarial emission. Among these, a specimen exposed at the age of 13 days showed parasitological cure, and three specimens were observed with sporocysts in the body tissues. The duration of the cercarial period in the Camorim colony was  $86.2 \pm 67.6$  days. In relation to Picos, 7 (11.7%) molluscs were anesthetized and fixed since they have stopped cercarial emission, although only one specimen exposed at the age of 13 days showed parasitological cure. The duration of the cercarial period in the Picos colony was  $53.6 \pm 55.87$  days.

In the *B. glabrata* colony, all the molluscs died of infection and never stopped cercarial emission; the duration of the cercarial period among the 81 molluscs that emitted cercariae and were observed until death was  $99.1 \pm 82.77$  days. According to the developmental stage, cercarial periods for immature and mature molluscs were, respectively:  $93.9 \pm 66.80$  days and  $32.4 \pm 45.67$  days for *B. straminea* from Camorim,  $67.9 \pm 59.93$  days and  $25.2 \pm 32.25$  days for *B. straminea* from Picos, and  $102.4 \pm 97.62$  days and  $95.4 \pm 62.99$  days for *B. glabrata* from Ressaca.

Overall cercarial emission for the three colonies was as follows: Camorim,  $14.8 \pm 14.76$  cercariae per mollusc per day; Picos,  $8.8 \pm 11.45$  cerc/mol/day; Ressaca,  $156.7 \pm 112.97$  cerc/mol/day. As regards sexual maturation, cercarial emission in the immature and mature molluscs, respectively, was as follows: Camorim,  $14.6 \pm 15.92$  cerc/mol/day and  $15.2 \pm 13.34$  cerc/mol/day, Picos,  $6.3 \pm 9.78$  cerc/mol/day and  $11.6 \pm 12.71$  cerc/mol/day, Ressaca,  $123.2 \pm 71.64$  cerc/mol/day and  $168.1 \pm 122.7$  cerc/mol/day. The data regarding cercarial emission by mollusc age are given in Table II.

Statistical analysis showed that: (1) significant associations were found between age of exposure and infection index in *B. straminea* (Camorim,  $\chi^2=163.0$ ,  $p<0.001$ ; Picos,  $\chi^2=27.4$ ,  $p<0.001$ ) as well as in *B. glabrata* ( $\chi^2=361.3$ ,  $p<0.001$ ). (2) Significant associations were also found between age of exposure and mortality index for the three colonies (*B. straminea* from Camorim,  $\chi^2=148.9$ ,  $p<0.001$ ; *B. straminea* from Picos,  $\chi^2=15.2$ ,  $p<0.05$ ; *B. glabrata*,  $\chi^2=17.9$ ,  $p<0.01$ ). (3) Significant differences were detected in the infection and mortality indices between immature and mature stages in *B. straminea* as well as in *B. glabrata* (infection and mortality, respectively: Camorim,  $\chi^2=63.83$ ,  $p<0.001$ ,  $\chi^2=65.3$ ,  $p<0.001$ ; Picos,  $\chi^2=17.18$ ,  $p<0.001$ ,  $\chi^2=5.1$ ,  $p<0.05$ ; Ressaca,  $\chi^2=243.7$ ,  $p<0.001$ ,  $\chi^2=7.5$ ,

$p<0.01$ ). (4) Significant differences were found in the infection index between *B. straminea* from Camorim and *B. glabrata* ( $Z=2.37$ ;  $p<0.05$ ), and between *B. straminea* from Picos and *B. glabrata* ( $Z=2.37$ ;  $p<0.05$ ), but not between *B. straminea* from Camorim and *B. straminea* from Picos ( $Z= -1.52$ ;  $p>0.05$ ). (5) No significant difference in the mortality index among the molluscs showing sporocysts, between *B. straminea* from Camorim and *B. glabrata* ( $Z=0.0$ ;  $p>0.05$ ), *B. straminea* from Picos and *B. glabrata* ( $Z=0.33$ ;  $p>0.05$ ), and *B. straminea* from Camorim and *B. straminea* from Picos ( $Z= -0.11$ ;  $p>0.05$ ). (6) On the other hand, among the dead molluscs that did not show sporocysts, a significant difference was detected between *B. straminea* from Camorim and *B. straminea* from Picos ( $Z= -2.06$ ;  $p<0.05$ ), but not between *B. straminea* from Camorim and *B. glabrata* ( $Z= -0.85$ ;  $p>0.05$ ) and *B. straminea* from Picos and *B. glabrata* ( $Z= 1.12$ ;  $p>0.05$ ). (7) Significant differences were found comparing the mortality index in each colony between positive and negative molluscs in *B. straminea* from Picos ( $Z= -2.20$ ;  $p<0.05$ ) and *B. glabrata* ( $Z= 1.99$ ;  $p< 0.05$ ), but not in *B. straminea* from Camorim ( $Z= -1.01$ ;  $p>0.05$ ). (8) A significantly negative correlation was found between infection index and mollusc diameter in *B. straminea* from Camorim ( $r= -0.844$ ;  $p<0.05$ ), but not in *B. straminea* from Picos ( $r= -0.684$ ;  $p>0.05$ ) or in *B. glabrata* ( $r= -0.660$ ;  $p>0.05$ ). (9) No significant correlation was detected between mortality index and mollusc diameter in *B. straminea* from Camorim ( $r=0.665$ ;  $p>0.05$ ), *B. straminea* from Picos ( $r=0.580$ ;  $p>0.05$ ) or *B. glabrata* ( $r=0.389$ ;  $p>0.05$ ). (10) Significant differences in the duration of precercarial period were found when comparing the mollusc age in the in *B. straminea* (Camorim,  $F=9.11$ ,  $p<0.001$ ; Picos,  $F=4.08$ ,  $p<0.01$ ) as well as in *B. glabrata* ( $F=4.51$ ,  $p<0.001$ ). (11) Significant differences were found in the duration of precercarial period between immature and mature molluscs (Camorim,  $F=30.09$ ,  $p<0.001$ ; Picos,  $F=5.07$ ,  $p<0.05$ ; Ressaca,  $F=11.70$ ,  $p<0.001$ ). (12) Significant differences were found in the precercarial period between *B. straminea* from Camorim and *B. straminea* from Picos (HSD=  $-0.033$ ;  $p<0.01$ ), and also between *B. straminea* from Camorim and *B. glabrata* (HSD =  $-0.056$ ;  $p<0.001$ ), but not between *B. straminea* from Picos and *B. glabrata* (HSD=  $-0.023$ ;  $p>0.05$ ). (13) Significant differences in the duration of cercarial period were found when comparing the mollusc age in the *B. straminea* colonies (Camorim,  $F=4.07$ ,  $p<0.001$ ; Picos,  $F=5.55$ ,  $p<0.001$ ), but not in *B. glabrata* ( $F=0.45$ ,  $p>0.05$ ). (14) No significant correlation was observed between the duration of cercarial period and the mollusc diameter at the time of the first cercarial emission (Camorim,  $r=0.002$ ,

TABLE II

Duration of cercarial period and rate of cercarial emission of *Biomphalaria straminea* and *Biomphalaria glabrata* exposed at the ages of 8, 13, 18, 21, 53, 83 or 114 days to sympatric *Schistosoma mansoni*

Colonies of molluscs/ Strains of <i>S. mansoni</i>	Sexual stage of snails	Age of snails (days)	NCP	Cercarial period (days) (mean±SE)	Shell diameter after 1 <sup>st</sup> cercarial emission (mean ± SE)	NEM	Cercarial emission (mean±SE)
<i>B. straminea</i> from Camorim (São Lourenço da Mata - PE)/ CM (Pitangueiras - PE)	Immature	8	40	101.1 ± 76.96	3.2 ± 1.06	6	4.5 ± 3.53
		13	52	74.2 ± 47.92	3.4 ± 0.67	4	12.4 ± 17.51
		18	17	98.3 ± 49.33	4.6 ± 0.88	0	-
		21	16	135.5 ± 87.94	5.8 ± 0.44	16	18.9 ± 17.19
	Mature	53	2	108.0 ± 132.94	7.8 ± 0.35	2	13.3 ± 10.82
		83	4	20.3 ± 25.38	8.0 ± 0.19	4	15.7 ± 17.15
		114	12	23.8 ± 16.37	7.9 ± 0.76	12	15.4 ± 13.54
<i>B. straminea</i> from Picos (Picos - PI)/ EC (Picos - PI)	Immature	8	2	4.5 ± 3.54	2.5 ± 0.71	2	1.5 ± 0.71
		13	10	79.7 ± 64.73	3.1 ± 0.86	1	19.5
		18	9	49.8 ± 19.49	4.6 ± 0.92	0	-
		21	19	76.8 ± 68.88	6.4 ± 0.70	19	6.1 ± 9.96
	Mature	53	7	24.4 ± 12.05	8.5 ± 0.41	7	8.4 ± 8.50
		83	1	52.0	9.0	1	3.6
		114	12	23.3 ± 40.59	9.3 ± 0.40	12	14.2 ± 14.83
<i>B. glabrata</i> from Ressaca (Belo Horizonte - MG)/ BH2 (Belo Horizonte - MG)	Immature	8	11	121.2 ± 132.26	3.9 ± 0.92	1	5.5
		13	11	74.0 ± 63.02	5.0 ± 1.29	1	34.7
		18	11	81.2 ± 62.52	7.5 ± 2.97	1	84.5
		21	10	136.4 ± 114.13	9.9 ± 1.23	10	147.8 ± 59.98
	Mature	53	12	107.2 ± 72.33	12.5 ± 2.92	12	98.7 ± 81.74
		83	13	100.7 ± 62.83	14.3 ± 1.01	13	214.5 ± 125.42
		114	13	79.3 ± 55.17	13.7 ± 1.38	13	185.8 ± 130.67

NCP; number of snails observed during of cercarial period; NEM: number of snails examined for cercarial emission.

p>0.05, Picos, r = - 0.152, p>0.05, Ressaca, r=0.216, p>0.05). (15) A significant difference in cercarial emission was found among the ages of exposure in *B. glabrata* (F= 2.89; p<0.05), but not in *B. straminea* (Camorim, F= 1.14, p>0.05; Picos, F= 1.46, p>0.05). (16) No significant difference was detected in cercarial emission between immature and mature stages (Camorim, F= 0.28, p>0.05; Picos, F= 3.96, p>0.05; Ressaca, F= 0.24, p>0.05). (17) Significant differences were found in cercarial emission between *B. straminea* from Camorim and *B. glabrata*

(HSD= 1.096; p<0.001) and also between *B. straminea* from Picos and *B. glabrata* from Ressaca (HSD= 1.407; p<0.001) and *B. straminea* from Camorim and *B. straminea* from Picos (HSD= -0.311; p<0.05).

### DISCUSSION

In the three colonies studied, susceptibility to *S. mansoni* varied with mollusc age at the time of exposure, the infection index being proportionally higher in immature molluscs than in mature ones.

This higher susceptibility in younger molluscs is not unexpected as it could be due to immune system functional immaturity, as pointed out by Dikkeboom et al. (1985), for *Lymnaea stagnalis*.

The overall infection indices found in the present work for *B. straminea* from Camorim (12.8%) and from Picos (5.8%), as well as for *B. glabrata* (68%), are in contrast with those found by other authors for colonies from the same geographical areas. Thus, Barbosa and Figueiredo (1970) obtained 0.6% for *B. straminea* from Camorim; Favre et al. (1995) found 0.5% for *B. straminea* from Picos; Souza et al. (1996) obtained 85.5% for *B. glabrata* from Ressaca. These discrepancies may be due to differences in methodology rather than in parasite-mollusc compatibility. The present infection indices were relatively lower in both colonies of *B. straminea* than in *B. glabrata*, confirming observations by Souza et al. (1995).

The infection indices obtained in the present study for *B. straminea* from Picos and *B. glabrata* from Ressaca can be statistically compared to those obtained by Fernandez (1997), because there were used the same methodologies. This author obtained an infection index of 71.5%, using *B. glabrata* from Ressaca at the age of 1, 2 and 3 months. The result was not significantly different ( $\chi^2 = 0.61$ ;  $p > 0.05$ ) from the one obtained in the present research concerning the same age in this colony. With the colony from Picos, Fernandez (1997) obtained an overall infection index of 9.7%, not significantly differing ( $\chi^2 = 3.05$ ;  $p > 0.05$ ) from the one obtained on this study.

The present results also show that only in *B. straminea* from Camorim the infection index differed with the diameters of the molluscs when exposed to miracidia. In *B. straminea* from Picos such difference was not detected, probably because the mean diameter ( $5.8 \pm 0.46$  mm) was statistically greater than the ones from Camorim ( $5.3 \pm 0.42$  mm) ( $F = 14.7$ ;  $p < 0.001$ ). In relation to *B. glabrata*, the lack of a correlation between size and infection might have been caused by the low index of infection (52.6%) in the molluscs exposed at the age of 8 days (1 mm of diameter). Therefore, if only the ages from 13 days on are considered (1.3 mm - 12.8 mm of diameter), a strong negative correlation is obtained ( $r = 0.96$ ;  $p < 0.01$ ). These data agree with that of Anderson et al. (1982) who, working with molluscs from 2 to 18 mm, observed a progressive decline on the infection index with increasing size.

The low infection index obtained in the *B. glabrata* molluscs exposed at the age of 8 days (Table I), comparing to the others, agrees with Cooper et al. (1992) who, working with molluscs at the ages of 4 to 70 days, obtained an infection index lower than 13% in the ones exposed at the age of 4

to 7 days ( $0.96 \pm 0.03$  mm), while in the other ages the index varied from 25 to 60%. These results show the necessity of including newly-born molluscs in experimental exposure studies, in order to obtain a more accurate estimate of the infection index.

The age of the mollusc on the exposure to miracidia influenced the mortality. In *B. straminea* from Picos and Camorim the mortality index was significantly higher for the mature ones, than for the immature. In *B. glabrata*, the highest index occurred with the mature ones that were free from infection, comparing to the others. However, Fernandez (1997) did not detect the influence of age on the mortality using *B. straminea* (molluscs exposed at the age of 30, 60 and 90 days). On the other hand, with *B. glabrata*, she verified the highest mortality index on the molluscs exposed at the age of 30 days. In the same way, Cooper et al. (1992), with *B. glabrata* from 4 to 41 days old obtained the highest mortality index on the molluscs that were 4 to 7 days old.

The mortality index in the three colonies studied here did not correlate with the diameter at the time of exposure. Barreto and Barbosa (1959) related mortality and mollusc diameter during the exposure to miracidia in *B. glabrata* from Pernambuco. According to these authors, molluscs measuring from 13 to 16 mm in diameter had a better survival, comparing to the ones measuring from 7 to 10 mm. However, this information cannot be compared to the ones in this study, for the considered diameter for *B. glabrata* varied from 1 to 13 mm.

Although these conflicts point to the need of more detailed studies in order to clarify the influence of *S. mansoni* infection on the survival of transmission species, the mortality indices obtained in this study were surprisingly low. If we take the mortality caused by infection into consideration, i.e., deducting that one obtained among the non-infected, there will be the following indices: 1.4% for *B. straminea* from Camorim, 7% for *B. straminea* from Picos and 0% for *B. glabrata* from Ressaca. Low mortality indices caused by infection were also obtained by Fernandez (1997) for *B. straminea* from Picos (0%) and *B. glabrata* from Ressaca (3.6%). These low indices are likely when the number of miracidia is somewhat small. Souza et al. (1995), using *B. glabrata* from Belo Horizonte exposed to *S. mansoni* sympatric, obtained a higher mortality index, comparing to the one obtained in this study, probably because the miracidian charge was 10 times higher than the one used in the present study.

The duration of the precercarial period is in accordance with those described by Favre et al. (1995) for *B. straminea* from Picos and *B. glabrata* from Belo Horizonte. In the three colonies, this period was influenced by the age of the mollusc. For

*B. straminea* from Camorim this period was significantly longer than in the other two colonies; a possible reason for that result is that the *S. mansoni* strain EC, used in *B. straminea* from Picos, and the BH2 strain, used in *B. glabrata* from Ressaca, are kept in laboratory for a substantially longer period, comparing to the CM strain, used in *B. straminea* from Camorim.

The presence of infected molluscs that do not shed cercariae might characterize a delay in the *S. mansoni* development. This fact was observed in a *B. glabrata* colony known by its resistance (Newton 1953), as well as in three other highly susceptible (Paraense & Corrêa 1963). According to these authors, there are individuals that react against the parasite, what may cause the precercarial period to be longer than expected.

The duration of the cercarial period was extremely variable in the three studied colonies, with the variation coefficient (standard deviation as a percentage of the mean) of 78.4% in *B. straminea* from Camorim, 104.3% in *B. straminea* from Picos and 83.6% in *B. glabrata* from Ressaca. High variation coefficients in the cercarial period were found in other studies on experimental infection. Thus, the results of Coelho and Barbosa (1956), for *B. straminea* from Pernambuco, revealed a variation coefficient of 122.7%, for a cercarial period of around 5.2 days. Similarly, Favre et al. (1995) showed a coefficient of 104.8% for *B. straminea* from Picos (cercarial period of 24.5 days) and a coefficient of 56.2% for *B. glabrata* from Belo Horizonte (31.2 days). This high variability may be the result of individual differences among the molluscs.

Parasitological cure was observed in the *B. straminea* colonies only, confirming the observations by Barbosa (1975) about the higher frequency of cure in *B. straminea* than in *B. glabrata*. It is interesting to notice that the cure index obtained by this author for *B. glabrata* from Northeast Brazil, did not differ significantly ( $\chi^2 = 0.05$ ;  $p > 0.05$ ) from the one obtained in the present work for that species.

The developmental stage of the mollusc when exposed to miracidia did not influence on the cercarial emission rate. These results, obtained here for the first time for *B. straminea*, agree with Gérard et al. (1993) in *B. glabrata*. These authors, studying the sporocysts population dynamics in relation to miracidian dose and host size, showed that the cercarial emission rate on the molluscs submitted to the same miracidian dose do not differ significantly between the immature and the mature.

For *B. glabrata* from Ressaca, the cercarial emission rate only differed significantly between the ages of 8 and 83 days (HSD = 1.44;  $p < 0.05$ ), and between 8 and 114 days (HSD = 1.41;  $p < 0.05$ ). The molluscs

exposed at the age of 8 days had a diameter of approximately 1 mm, whereas the ones exposed at the ages of 83 and 114 days measured more than 11 mm. These results are compatible with the ones found by Niemann and Lewis (1990) who, working with *B. glabrata* from Puerto Rico, observed that molluscs exposed when measuring more than 11 mm eliminated a significantly higher proportion of cercariae, than the smaller ones. The significantly lower number of emitted cercariae in *B. straminea* than in *B. glabrata* obtained in the present work confirm the findings by Souza et al. (1983).

This work reinforces the view that the age of the mollusc when exposed to the parasite is an important variable and should be taken in to consideration in studies of parasite-mollusc compatibility. Considering that the usual methods for collecting molluscs tend to overlook those under 2 mm in shell diameter (Olivier & Uemura 1973), estimates of mollusc infection in natural habitats may be underrated. Therefore, particular attention should be given to the smaller, younger molluscs when sampling natural populations.

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