

***Biomphalaria tenagophila* (ORBIGNY, 1835) (MOLLUSCA): ADAPTATION TO DESICCATION AND SUSCEPTIBILITY TO INFECTION WITH *Schistosoma mansoni* SAMBON, 1907**

Fernanda Pires OHLWEILER(1) & Toshie KAWANO(1)

SUMMARY

Experiments were carried out to test the susceptibility of *Biomphalaria tenagophila* to the infection with strain SJ of *Schistosoma mansoni* in the F₁, F₂ and non-selected parental generation. The potential adaptation of *B. tenagophila* to desiccation, in healthy mollusks and those exposed to the larvae of *S. mansoni* of the F₁, F₂ and non-selected parental generations was also studied. The presence of mucus and soil, at the shell opening, protected the snails against desiccation, favoring survival. The healthy mollusks performed more attempts against desiccation than those exposed to the larvae of the parasite. The mortality rate, during desiccation, was higher among mollusks that remained buried and with the shell opening unobstructed. During the desiccation period the stage of development of the parasite was influenced by the weight loss and the survival of the snails. The longer the period of desiccation, the greater was the weight loss observed, abbreviating survival. The non-selected parental generation was more sensitive to desiccation than the F₁ and F₂ generations, both in healthy mollusks and in those exposed to *S. mansoni* larvae. Healthy mollusks were more resistant to desiccation than those exposed to the larvae of the *S. mansoni*. Desiccation did not interrupt the development of *S. mansoni* larvae in mollusks, causing a delay in the cercariae elimination. The susceptibility of *B. tenagophila* to the SJ strain of *S. mansoni*, in mollusks maintained in water during the larvae incubation period, was similar in all three generations.

KEYWORDS: *Biomphalaria tenagophila*; *Schistosoma mansoni*; Desiccation and susceptibility.

INTRODUCTION

Biomphalaria tenagophila (Orbigny, 1835) is responsible for the propagation of schistosomiasis in the states of Rio de Janeiro, Minas Gerais, São Paulo and Santa Catarina²¹. Several authors have studied the susceptibility of *B. tenagophila* to the infection by *S. mansoni*. Most of them observed a low or even null susceptibility of this species to the infection by the parasite^{13,14,31,32,44,47}.

There are several variations in the degree of susceptibility of *B. tenagophila* to the infection by *S. mansoni*, although there is still a predominance of refractory populations over susceptible populations⁹.

Adaptation to adverse environmental conditions is an important aspect of *Biomphalaria*, since these mollusks are easily dispersed. Studies on the resistance to desiccation were carried out on healthy specimens of *B. glabrata*, *B. straminea* and *B. pfeifferi* by BARBOSA & DOBBIN (1952)⁷, OLIVIER & BARBOSA (1955, 1956)^{24,25}, PIERI *et al.* (1980)³⁸, VIANEY-LIAUD & LANCASTRE (1986 a and b)^{50,51}, VIANEY-LIAUD & DUSSART (1994)⁵² and JONG-BRINK (1973)²⁰. These studies were also carried out on mollusks infected by *S. mansoni* by BARBOSA (1953, 1959)^{2,3}, BARBOSA & COELHO (1953, 1955)^{5,6}, BARBOSA & BARBOSA (1958)⁴, LANCASTRE *et al.* (1987, 1989)^{22,23} and BADGER

& OYERINDE (1996)¹. For *B. tenagophila*, only TELES & MARQUES (1989)⁴⁹, are cited. These authors observed healthy specimens in natural desiccation conditions, in Ubatuba and Conchas, state of São Paulo.

The purpose of this work was to study F₁ and F₂ generations of *B. tenagophila*, as well as parental generations, in relation to their susceptibility to the infection by *S. mansoni* and their adaptation to desiccation.

MATERIAL AND METHODS

The *B. tenagophila* mollusks (7 mm to 10 mm shell diameter) descend from specimens collected in Florianópolis, state of Santa Catarina, Brazil. Cross-breedings were made between melanic mollusks (selected parental generation, susceptible to *S. mansoni*) and albino mollusks (selected parental generation, refractory to *S. mansoni*) resulting in a melanic F₁ generation. A melanic F₂ generation was obtained by auto fecundation. The F₁ generation mollusks were obtained from the eggmass of albino specimens, used as markers. Melanic mollusks of the parental generation, non-selected for the susceptibility character, were also used in the experiments. The *S. mansoni* strain is from São José dos Campos (SJ), state of São Paulo. The parental, F₁ and F₂ generations of non-selected mollusks were exposed, to 10 miracidia of *S. mansoni*, for a period of 4

(1) Instituto Butantan, Laboratório de Parasitologia, Av. Vital Brasil 1500. 05503-900 São Paulo, SP, Brasil.

Correspondence to: Dra. Fernanda Pires Ohlweiler, e-mail: fernpi@terra.com.br.

hours each. Miracidia were obtained from the eggs of a hamster's liver, according to the technique proposed by PELLEGRINO & KATZ (1968)³⁴.

Desiccation experiments were carried out according to the technique proposed by OHLWEILER & KAWANO (2001)²⁶. Soil humidity was obtained by means of the weighing method, in which 3 soil samples, one from each recipient, were weighed on the first and last days of the desiccation process. By means of the average weight of each recipient, the quantity of evaporated water, during each desiccation period, was obtained.

Healthy mollusks were subjected to desiccation for 7, 14, 21 and 28 days. Twenty mollusks from each group were used for each generation. The desiccation experiments with mollusks exposed to *S. mansoni* miracidia were divided in 5 groups, as shown in Fig. 1. A total of 415 exposed mollusks were desiccated. Of these, 126 were from the F₁ generation (28 from group I, 20 from group II, 38 from group III, 20 from group IV and 20 from group V), 126 from the F₂ generation (20 from group I, 20 from group II, 40 from group III, 20 from group IV and 26 from group V) and 163 from the non-selected parental generation (34 from group I, 20 from group II, 24 from group III, 40 from group IV and 45 from group V). Mollusks were weighed before and after desiccation. The difference between both measures supplied the weight loss for each mollusk, in each desiccation period.

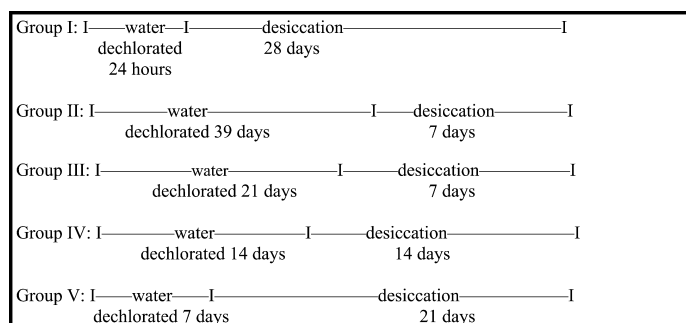


Fig. 1 - Five experimental groups for *Biomphalaria tenagophila* maintained in water after being exposed to *Schistosoma mansoni* miracidia.

To study the susceptibility of *B. tenagophila* to the infection by *S. mansoni*, 105 mollusks from the non-selected parental generation, 143 mollusks from the F₁ generation and 100 mollusks from the F₂ generation, were used.

The statistical analysis followed BOX *et al.* (1978)¹⁰ and

FIENBERGS (1989)¹⁸. The study of the relationship between weight loss, desiccation period and soil humidity was obtained by means of logistical regression and χ^2 test. The relationship between mollusk weight loss and the different desiccation periods, among the generations, was studied by means of a variance analysis (Tukey test). To compare healthy mollusks with those exposed to the parasites' miracidia, for each desiccation period, χ^2 test was used for survival rates and T-test for weight loss. For all tests and statistical analysis p value was used as an indicator of significant evidence. If $p < 0.01$, the evidence is highly significant; between 0.01 and 0.05 significant; between 0.05 and 0.10 evidence is only slightly significant and above 0.10 evidence is non significant.

RESULTS AND DISCUSSION

Generally, mollusks surface during the desiccation period, when the earth is soft and slightly moist. As the earth began drying out, mollusk movements decreased. Others remained partially or completely buried during all the desiccation period. Some healthy mollusks, when submitted to desiccation, dug tunnels up to the surface, by means of which they maintained contact with the exterior (Table 1). PIERI *et al.* (1980)³⁸ observed similar behavior in *B. glabrata* specimens. According to these authors, the mollusks would surface for gaseous exchange and, not rarely, re-bury themselves.

When removed from desiccation, some mollusks presented whitish mucus or soil around the shell opening. Others presented a completely unobstructed opening. On healthy mollusks a higher percentage of mucus secretion or soil was observed, while in infected mollusks the higher percentage was for soil or unobstructed shell openings (Table 2).

Mollusks with soil around the shell openings probably left their shells and returned later, causing the soil to adhere to the mucus around the opening. The soil acts as a protection against desiccation, as verified by PIERI *et al.* (1980)³⁸ for *B. glabrata*. Mollusks that retract inside the shell offer less exposed surface area, diminishing the effect of environmental adversities³⁵. Mollusks that secreted mucus at the shell opening (forming a kind of epiphragma), were certainly trying to protect themselves against desiccation. Those with completely unobstructed shell openings probably did not try to protect themselves. Healthy mollusks performed more protection trials than those infected.

The percentages of mollusks, both healthy and infected, that died during desiccation, that surfaced or, that remained partially or completely buried, as well as the percentages of mollusks that, when removed from

Table 1

Biomphalaria tenagophila. F₁, F₂ and non-selected parental generations. Average percentages of healthy and infected mollusks that surfaced, dug tunnels, remained partially or completely buried, during desiccation

Generations		Completely buried %	Partially buried %	tunnels %	% surfaced on the				
					1 st day	2 nd day	3 rd day	4 th day	5 th day
F ₁	healthy	25.0	1.2	0	0	0	73.7	0	0
	infected	8.73	0	0	3.97	4.76	75.40	7.14	0
F ₂	healthy	17.5	1.2	5.0	0	0	68.7	6.2	1.2
	infected	8.73	0.79	0	5.56	7.94	53.97	23.01	0
parental	healthy	34.5	0	0	0	25.9	39.5	0	0
	infected	18.40	1.84	0	24.54	29.45	25.77	0	0

Table 2

Biomphalaria tenagophila. F₁, F₂ and non-selected parental generations. Average percentages of healthy and infected mollusks that presented whitish mucus or soil at the shell opening or unobstructed shell openings, after removed from desiccation

Generations		Agglomerated soil %	Whitish mucus %	Unobstructed shell opening %
F ₁	healthy	21.2	66.2	12.5
	infected	49.2	33.3	17.5
F ₂	healthy	26.2	57.5	16.3
	infected	52.4	19.0	28.6
parental	healthy	43.2	18.5	38.3
	infected	32.5	18.0	50.3

the soil, presented the shell opening unobstructed or covered by soil or mucus, are shown on tables 3 to 5. Mortality rates for healthy mollusks were higher among those that remained buried, while for the infested mollusks, mortality rates were high not only among those that remained buried but also among those that surfaced.

OLIVIER & BARBOSA (1956)²⁵ state that, in the field, *B. glabrata* and *B. straminea* survival rates are higher amongst those that remain buried than amongst those that surface. This can be explained by the

Table 3

Biomphalaria tenagophila. F₁ and F₂ generations. Percentages of healthy mollusks, that did not resist desiccation, that surfaced, remained partially or completely buried during desiccation and that when removed from the earth presented unobstructed shell openings

Healthy generations	Desiccation	Unobstructed shell openings %		
		Surfaced	Remained completely buried	Remained partially buried
F ₁	07 days	0	0	0
	14 days	5	0	0
	21 days	0	10	0
F ₂	28 days	0	30	5
	07 days	0	10	0
	14 days	5	0	0
	21 days	0	15	0
	28 days	0	35	0

Table 4

Biomphalaria tenagophila. Non-selected parental generation. Percentage of healthy mollusks, that didn't resist desiccation, that surfaced, that remained partially or completely buried during desiccation and that, when removed from the earth, presented unobstructed shell openings

Healthy generation	Desiccation	Surfaced %	Remained buried %	Shell opening %	
				unobstructed	agglomerated earth
parental	07 days	0	15.0	15.0	0
	14 days	10.0	20.0	25.0	5.0
	21 days	55.0	20.0	55.0	20.0
	28 days	19.0	57.1	57.1	19.0

Table 5

Biomphalaria tenagophila F₁, F₂ and non-selected parental generations. Groups I through V. Percentage of mollusks that did not resist desiccation, that surfaced during desiccation and, when removed from the earth, presented unobstructed shell openings or with earth at the shell opening

Group	Generation	Surfaced %	Remained buried %	Shell opening %		
				unobstructed	earth	mucus
I	F1	32.1	0	0	32.1	0
	F2	55.0	0	45.0	10.0	0
	parental	88.2	0	52.9	35.3	0
II	F1	65.0	0	25.0	40.0	0
	F2	45.0	5.0	15.0	30.0	5.0
	Parental	30.0	50.0	65.0	15.0	0
III	F1	10.5	21.0	31.6	0	0
	F2	42.5	0	12.5	17.5	12.5
	parental	58.3	4.2	37.5	16.7	8.3
IV	F1	35.0	0	0	0	35.0
	F2	10.0	35.0	40.0	5.0	0
	parental	30.0	27.5	42.5	15.0	0
V	F1	15.0	10.0	25.0	0	0
	F2	46.1	0	11.5	26.9	7.7
	parental	36.6	15.5	53.3	8.9	0

fact that, in natural environments, soil layers are moister. In our experiments, the quantity of earth is smaller than in natural environments. Consequently, water evaporation is faster and more homogeneous throughout the soil sample. In this case, the lack of humidity in the soil propitiates an unfavorable environment for the mollusks, contributing to mortality, especially in those that remain buried. The mollusks that remained buried, during desiccation, probably lacked the strength to surface, showing more weakness towards the unfavorable environmental conditions to which they were exposed.

The healthy mollusks, from generations F_1 and F_2 , and the majority of those of the non-selected parental generation, that died during desiccation, presented unobstructed shell openings. In infected mollusks, mortality rates were higher in those that presented unobstructed shell openings. This is due to the fact that the mollusks were more exposed to environmental adversities; being subject to greater water loss from the tissues. Some sweet water mollusks can protect themselves from environmental adversities by means of other mechanisms, such as an operculum and lamella formations. CORT (1920)¹² states that the presence of an operculum enables the opening and closing of the shell, protecting mollusks against water loss. Conversely to Cort's observations, BARLOW (1935)⁸ states that mollusks do not have to be operculated to protect themselves against water loss. This is corroborated by our experiments with *B. tenagophila*. Lamella formations at the shell opening were observed by PARAENSE (1957)²⁷, RICHARDS (1967 and 1968)^{39,40}, PIERI & THOMAS (1986 and 1992)^{36,37} and DANNEMAN & PIERI (1993)¹⁶ for *B. glabrata* specimens kept out of water. These authors believe that the capacity to form lamella is a genetically inherited character. Lamella formations were not observed in our experiments with *B. tenagophila*.

The survival rates, weight loss during desiccation, earth humidity and evaporated water from the soil are demonstrated in Fig. 2 to 21.

In healthy mollusks the drop in survival rates is only slightly significant for generation F_2 ($p = 0.0545$); significant for generation F_1 ($p = 0.0041$) and highly significant for the non-selected parental generation ($p = 0.00004$). The behavior is similar for the F_1 and F_2 generations, differing from the non-selected parental generation (Fig. 2). For infected mollusks, survival rates are higher for the generation F_1 (except in group II), followed by generation F_2 and by the non-selected parental generations, the latter with the lowest survival rates (group I: $p = 0.000064$; group II: $p = 0.138346$; group III: $p = 0.056557$; group IV: $p = 0.240268$ and group V: $p = 0.019911$) (Fig. 3). If we add groups II and III (7 desiccation days), survival rates for generation F_1 is the highest for all periods, followed by generation F_2 and by the non-selected parental generation.

Figures 4 to 6 show that, in healthy mollusks, weight loss increases with the increase of the desiccation period, for all three generations. Despite weight loss having been the same for all three generations, in the 7 day desiccation period, the variation pattern (for 07, 14, 21 and 28 days) is similar for generations F_1 and F_2 , differing only for the non-selected parental generation. A significant effect of the desiccation period and generation was observed for weight loss ($p < 0.00001$). In generation F_1 , only mollusks that presented less than 50% weight loss survived ($p = 0.000$). In generation F_2 , mollusks that lost more than 50% of their initial weight, as well as a few that lost less than 50% of the initial weight, died ($p = 0.000$). In the non-selected parental generation, most mollusks that died during desiccation lost more than 50% of their initial weight and

only a few lost less than 50% of their initial weight. There were a few cases of mollusks that, despite losing more than 50% of their initial weight, survived desiccation ($p = 0.000$). This shows that there is indeed a high correlation between weight loss and survival rates for healthy mollusks. Although the desiccation period and soil humidity have no significant influence on the survival rate of healthy mollusks, they are greatly correlated to the weight loss.

As shown on Fig. 7 to 9, weight loss amongst infected mollusks is higher for the non-selected parental generation than for generations F_1 and F_2 . The weight loss behaviour for generations F_1 and F_2 is similar, differing from the non-selected parental generation. In the parental non-selected generation, weight loss is greater in groups I and II, while in generations F_1 and F_2 , it is greater in groups I and V.

In group I, before desiccation, the mollusks were infected by larvae in the miracidia stage. These, generally, do not cause great damage. We believe that the greater weight loss of mollusks from group I was caused by the 28 day desiccation period and not by the infection, that could have caused lower survival rates for the non-selected parental generation and for generation F_2 . In this group, generation F_1 was more resistant (Fig. 3).

In group II, before desiccation, mollusks were infected by larvae in more advanced stages (sporocysts II and cercariae), that compete for the mollusks nutrient reserves, leaving them more debilitated, and subject to greater weight loss. In this group, survival rates were low for all three generations (Fig. 3). The advanced development stage of the *S. mansoni* larvae had more influence over mollusks survival rates than the 7 day desiccation period.

In group III, weight loss was more influenced by the development stage of the larvae (sporocysts II) than by the desiccation period (7 days).

In group IV, weight loss is associated to the larvae development stage (sporocysts II) as well as the desiccation period (14 days).

In group V, before desiccation, mollusks were infected by larvae in an initial development stage (sporocysts I), causing little damage. We believe that the 21 day desiccation period leaves mollusks more vulnerable to weight loss, as observed in generations F_1 and F_2 . In the non-selected parental generation, weight loss in group V was also great, being even greater than in generation F_1 .

We verified that in infected mollusks from the non-selected parental generation there is a relation between weight loss and survival rate ($p = 0.000001$). The same was not observed for the soil humidity ($p = 0.83257$). The variable desiccation period, despite having little significance, can still afford some explanation ($p = 0.06045$). In generation F_2 , weight loss ($p = 0.00001$), soil relative humidity ($p = 0.01735$) and desiccation period ($p = 0.00089$) are significant variables for survival rate probability in mollusks. In generation F_1 , weight loss ($p = 0.000001$) and desiccation period ($p = 0.00089$) are significant variables for survival rate probability in mollusks. The significant difference between weight loss and the different desiccation groups (I to V), for all generations, can be attributed not only to desiccation, but also to the different development stages of the parasite's larvae, in each desiccation group, causing mollusks to be more or less vulnerable.

From the comparative study of healthy and infected mollusks, we observed that, for 07 and 14 days, the survival rates were significantly higher for healthy mollusks than for those of groups II, III and IV (F_1 : $p = 0.0000610$; F_2 : $p = 0.0159549$; non-selected parental generation: $p = 0.0001050$). For 21 days, survival rates were higher for healthy mollusks than for those of group V in the F_1 and F_2 generations. For the non-selected parental generation survival rates are lower for healthy mollusks. The difference is significant for generation F_2 and non significant for generation F_1 and for the non-selected parental generation (F_1 : $p = 0.211958$; F_2 : $p = 0.025450$; non-selected parental generation: $p = 0.314903$). For 28 desiccation days, we observed that survival rates were higher for healthy mollusks than for group I mollusks, in the non-selected parental generation and in the F_2 generation. For group I mollusks of the generation F_1 , survival rates are similar to those observed for healthy mollusks from the 28 day desiccation period. Nevertheless, for all generations the difference is not significant (F_1 : $p = 0.835725$; F_2 : $p = 0.203650$; non-selected parental generation: $p = 0.240784$) (Fig. 2 and 3).

Weight loss is greater in healthy mollusks exposed to a 28 desiccation period than for those in group I. The difference is significant for generation F_1 ($p = 0.0029$) and non significant for generation F_2 ($p = 0.99$). For the non-selected parental generation the difference is in the limit between significant and little significant – the evidence is too weak ($p = 0.051$). Weight loss, in healthy mollusks exposed to a 07 day desiccation period, is smaller than in groups II and III mollusks. The

difference is not very significant for generation F_1 ($p = 0.078$), non-significant for generation F_2 ($p = 0.32$) and significant for the non-selected parental generation - where the evidence is very strong ($p < 0.00001$). Weight loss for healthy mollusks exposed to a 14 day desiccation period is smaller than that for group IV mollusks. The difference is significant for generation F_1 ($p = 0.030$) and for generation F_2 ($p = 0.0032$), and non significant for the non-selected parental generation ($p = 0.54$). Weight loss in healthy mollusks, exposed for a 21 day desiccation period, is smaller than that for group V mollusks, only in generation F_2 . In the non-selected parental generation there is an accentuated weight loss in healthy mollusks. For the generation F_1 , weight loss is very small. The difference between the weight loss in healthy mollusks and in group V mollusks is significant for generation F_2 ($p = 0.0065$), non-significant for generation F_1 ($p = 0.80$) and highly significant for the non-selected parental generation ($p < 0.00001$) (Fig. 4 to 9).

Desiccation period and relative soil humidity do not have a significant influence on the survival rates of healthy mollusks. Nevertheless, they are correlated to the weight loss. As the desiccation period increases, the soil's relative humidity diminishes, increasing mollusk weight loss. In this case, the larger the weight loss, the lower the survival rate for mollusks (Fig. 16 to 21).

For the infected mollusks, the desiccation period had a significant influence on mollusk survival rates. This was also observed by VIANEY-LIAUD & DUSSART (1994)⁵² for *B. glabrata* infected by *S. mansoni*. The desiccation period, together with the parasite larvae, debilitate the mollusks that are then subject to larger weight loss. As observed for healthy mollusks, the increase of the desiccation period diminished soil humidity, increasing weight loss and reducing chances of survival.

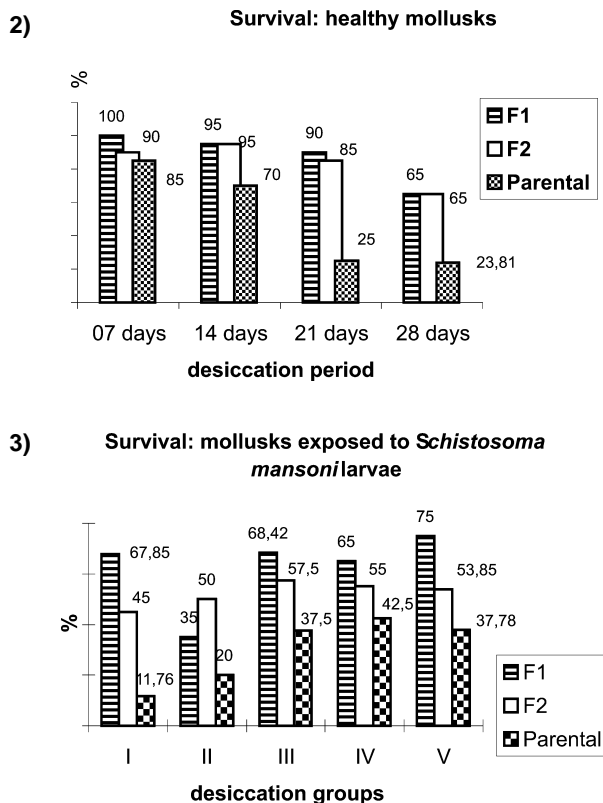
Desiccation causes a drop in mollusk metabolism, leaving them more debilitated. During the beginning of the desiccation period, the mollusks still maintain some locomotor activity, causing energy waste. The larger the energy waste, the larger the weight loss. Nevertheless, in the beginning of the desiccation period, the earth is still relatively humid, favoring oxygenation and humidity of mollusk tissues, slightly compensating weight loss caused by locomotor activity.

According to OLIVIER & BARBOSA (1956)²⁵ and PIERI *et al.* (1980)³⁸, soil humidity, favoring water retention in mollusk tissue, is a primary factor for mollusk survival under desiccation conditions. VIANEY-LIAUD & LANCASTRE (1986 b)⁵¹ also support the theory that the survival of desiccated mollusks is longer in a saturated atmosphere. According to these authors, the water deficit is more important for mollusk survival than nourishment. They believe that lack of water can cause death before starvation can have any effect.

Despite the low locomotor activity, presented by the mollusks during desiccation, these also dispend energy due to their physiological necessities, causing weight loss, which increases mortality rates.

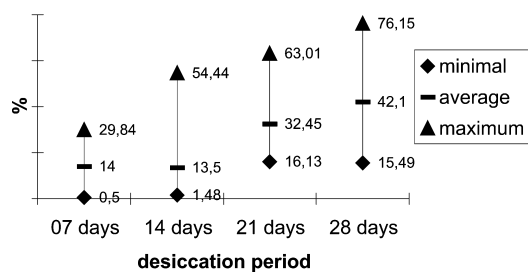
We agree with OLIVIER & BARBOSA (1956)²⁵, PIERI *et al.* (1980)³⁸ and VIANEY-LIAUD & LANCASTRE (1986 a, b)^{50,51}, in that the lower the soil humidity, the greater will be the water deficit in mollusk tissues; consequently, weight loss will be greater and survival abbreviated.

In infected mollusks, besides the above discussed factors and the stress

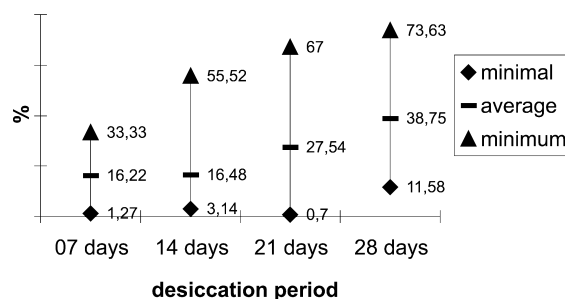


Figs. 2 and 3 - Survival index for *Biomphalaria tenagophila* from the F_1 , F_2 and non-selected parental generations. **2** - Healthy mollusks desiccated for 7, 14, 21 and 28 days. **3** - Mollusks exposed to *Schistosoma mansoni* larvae, groups I through V.

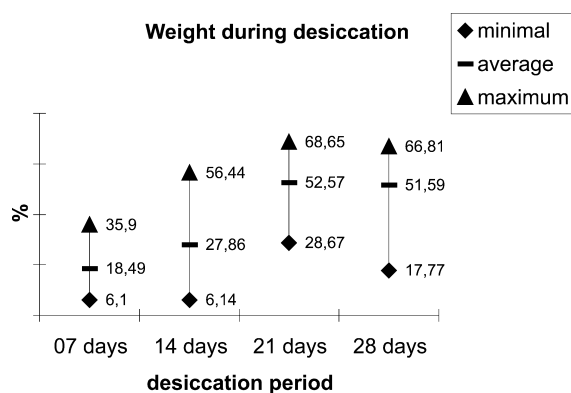
4) Weight loss during desiccation



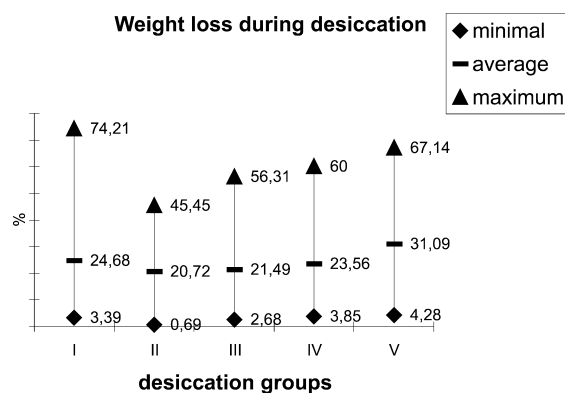
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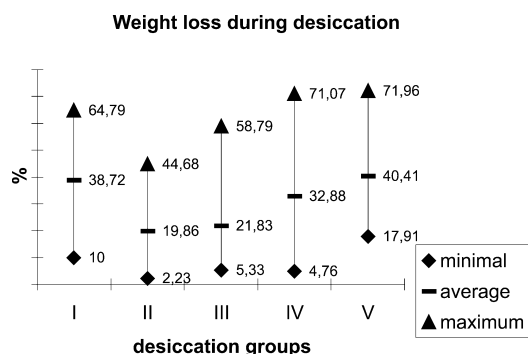
6) Weight during desiccation



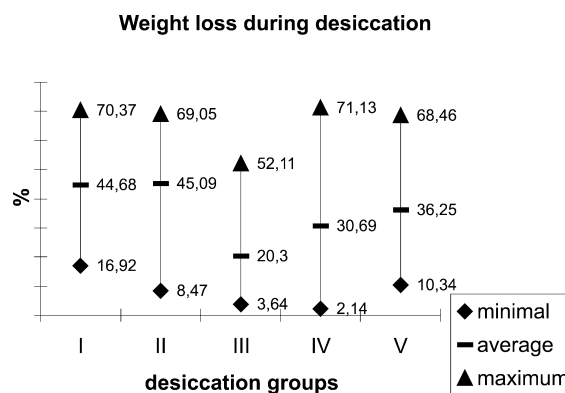
7) Weight loss during desiccation



8) Weight loss during desiccation



9) Weight loss during desiccation



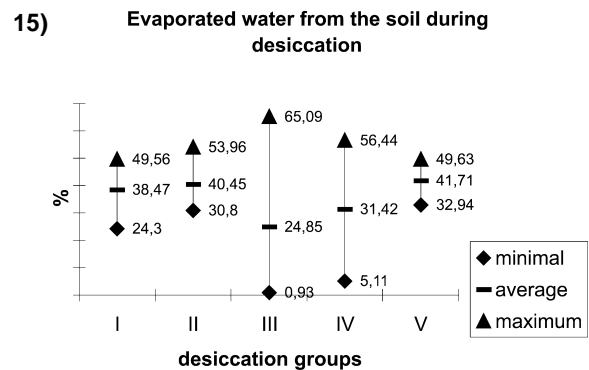
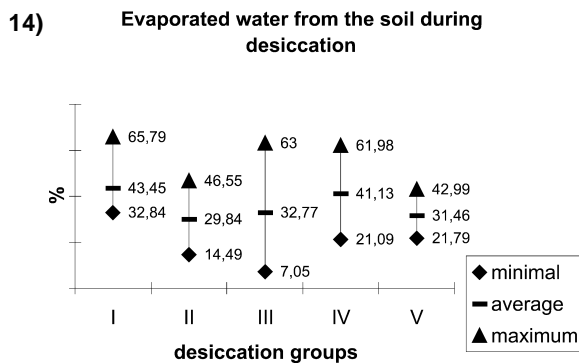
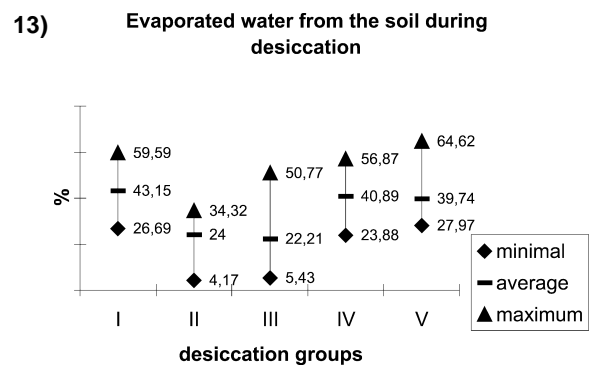
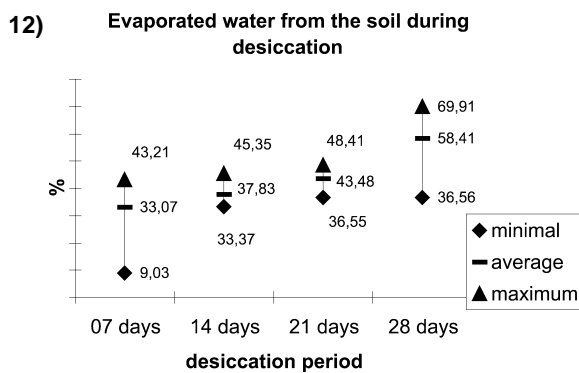
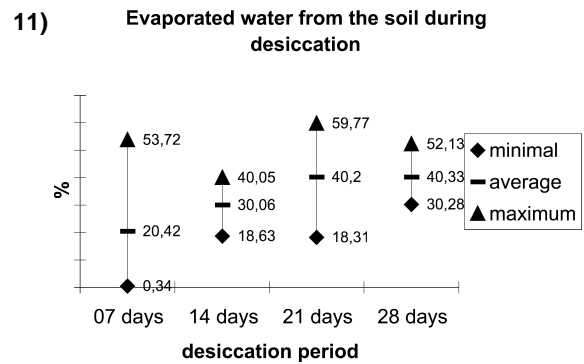
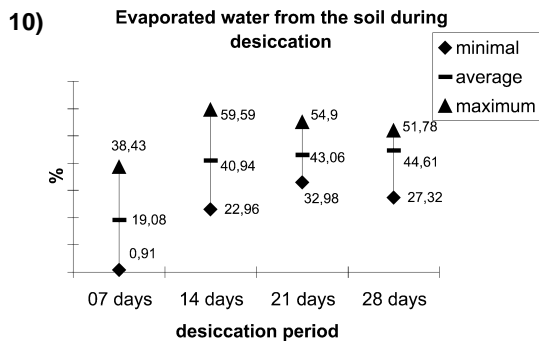
Figs. 4 to 9 - Weight loss for *Biomphalaria tenagophila* from the F₁, F₂ and non-selected parental generations. 4-6 - Healthy mollusks desiccated for 7, 14, 21 and 28 days. 7-9 - Mollusks exposed to *Schistosoma mansoni* larvae, groups I through V. 4, 7 - F₁ generation. 5, 8 - F₂ generation. 6, 9 - Non-selected parental generation.

suffered during desiccation – that also affects healthy mollusks – the development stage of the parasite’s larvae also has great influence on survival rates. The physical resistance of each mollusk is also an influential factor and varies from specimen to specimen within each species.

Tables 6 and 7 show the data of the observations carried out shortly after the mollusks were replaced in water. In groups I through V, the time that the mollusks took to begin eliminating cercariae, after desiccation, was larger or remained the same as that of the period in which they were under desiccation. In group II, 5% of the mollusks of

the non-selected parental generation no eliminated cercariae when placed back in the water showing that desiccation interrupted the parasites’ cycle in these mollusks. In group III from generation F₂, there was no cercariae elimination after the mollusks were returned to water. This does not mean that larvae cannot develop inside desiccated mollusks from this generation. Larvae with 21 days were more sensitive to desiccation also in the non-selected parental generation and in generation F₁, although they were still present.

Susceptibility of *Biomphalaria tenagophila* exposed to *Schistosoma*



Figs. 10 to 15 - Evaporated water from the soil. **10-12** - During 7, 14, 21 and 28 desiccation days. **13-15** - Groups I through V. **10** - Healthy F₁ generation. **11** - Healthy F₂ generation. **12** - Healthy non-selected parental generation. **13** - F₁ generation exposed to *Schistosoma mansoni* larvae. **14** - F₂ generation exposed to *Schistosoma mansoni* larvae. **15** - Non selected parental generation exposed to *Schistosoma mansoni* larvae.

***mansoni* larvae, maintained in water during the incubation period of the larvae:** The susceptibility index for mollusks exposed to *S. mansoni* larvae was 32.17% in generation F₁, 36.06% in generation F₂ and 38.10% in the non-selected parental generation. There was no significant difference for susceptibility between generations ($p = 0.8164$)

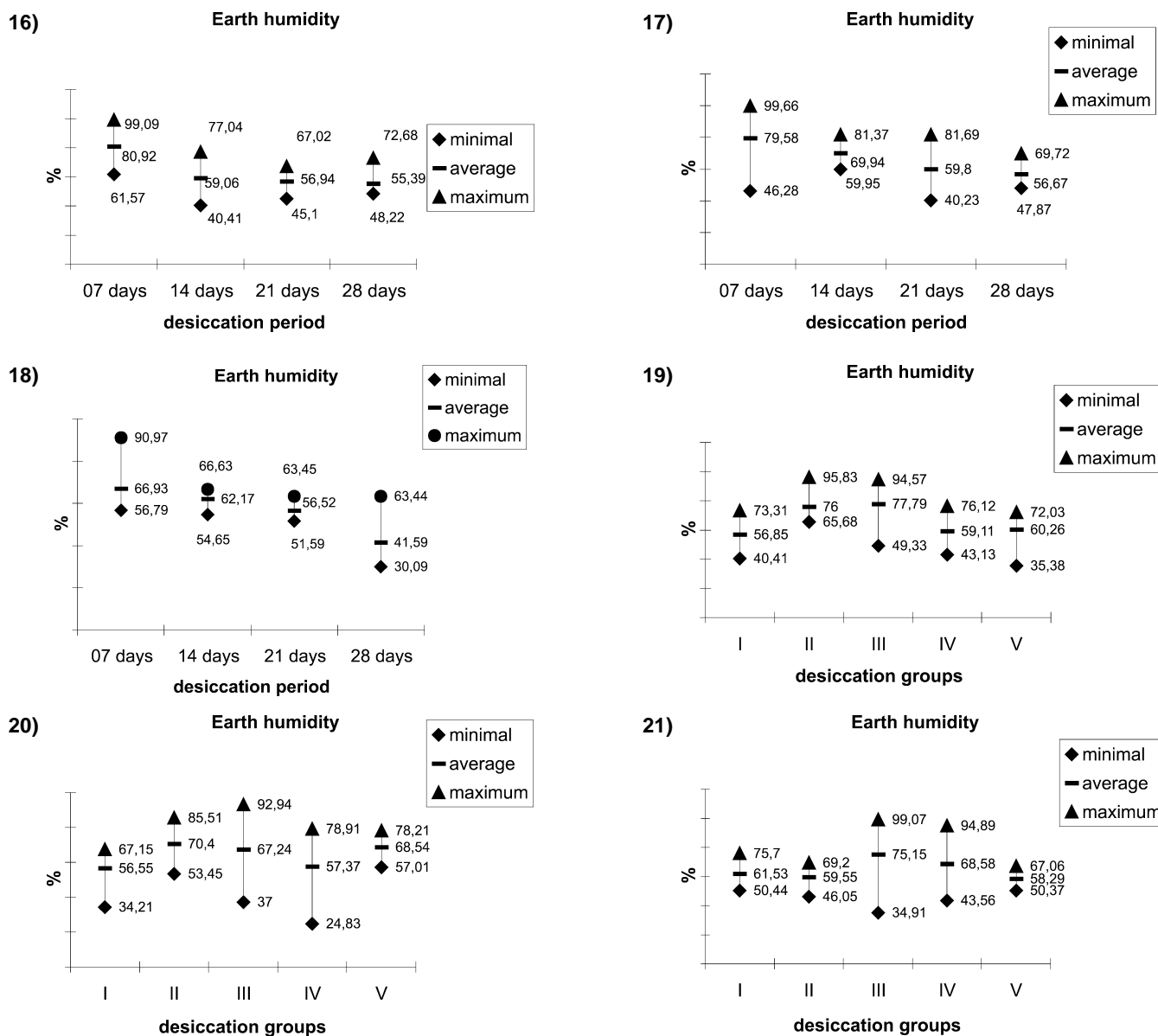
The susceptibility index supplied by PARAENSE & CORRÊA (1982)³¹, for *B. tenagophila* – also from Santa Catarina and infected with *S. mansoni* SJ strain – was lower (17.9%) than the index obtained in our studies.

According to PARAENSE & CORRÊA (1963 b)²⁹, while breeding refractory mollusks with susceptible ones, it is possible to obtain hybrids with intermediate susceptibility indexes in relation to the parental strain.

The genetic constitution of the species involved in the infection (host and parasite) is inherited through generations^{19,29,53}.

Considering susceptibility a genetically inherited factor, RICHARDS & MERRITT (1972)⁴¹ state that susceptibility genes can be transmitted by refractory mollusks, and genes for the non susceptible character can be transmitted by susceptible mollusks. This is corroborated by SANTANA *et al.* (1978)⁴³, who obtained variations on the susceptibility degree for *B. tenagophila* and *B. glabrata*, exposed to *S. mansoni* larvae, by means of selection, verifying that mollusk susceptibility increased as of generation F₁.

In the experiments with *B. tenagophila*, the susceptibility indexes in mollusks, increased from generation F₁ to generation F₂. The difference



Figs. 16 to 21 - Relative soil humidity. **16-18** - After 7, 14, 21 and 28 desiccation days. **19-21** - Groups I through V. **16** - Healthy F₁ generation. **17** - Healthy F₂ generation. **18** - Healthy non-selected parental generation. **19** - F₁ generation exposed to *Schistosoma mansoni* larvae. **20** - F₂ generation exposed to *Schistosoma mansoni* larvae. **21** - Non-selected parental generation exposed to *Schistosoma mansoni* larvae.

existing between them is non significant, as with the non-selected parental generation.

It is believed that the non-selected parental generation carries genes for the susceptible and refractory characters, which were also inherited by generations F₁ and F₂, from the selected parental generation (refractory albino and susceptible melanic).

The mollusk mortality rate, during the larvae incubation period, was 23.78% in generation F₁, 6.56% in generation F₂ and 11.43% in the non-selected parental generation. There is a significant difference as to

mortality among the three generations (p = 0.0007).

Mortality rate, during the larvae incubation period, is higher in generation F₁, followed by the non-selected parental generation and by the generation F₂. The difference is statistically significant for the three generations.

The generation F₁ seems to be more refractory towards the infection by the parasite's larvae than the non-selected parental generation and generation F₂, since it's mortality rates were higher, the susceptibility indexes lower (although the difference is non significant) and the

parasite's life cycle longer (Table 8).

The susceptibility indexes for *B. tenagophila* are lower than those presented, in the literature, for *B. glabrata* (approximately 50% to 96%) and, generally higher than those obtained for *B. straminea* (from 31% to 24%)^{11,15,17,28,30,33,42,45,46,48}.

CONCLUSIONS

Based on the data observed we conclude that the presence of mucus and soil at the shell opening protected the mollusks against desiccation, favoring survival; healthy mollusks attempted to protect themselves more against desiccation than those exposed to the parasite's larvae; mortality, during desiccation, was higher among those mollusks that remained buried and presented unobstructed shell openings; the development stage of the parasite's larvae, during desiccation, influenced mollusk weight loss and survival rates; the longer the desiccation period, the greater the weight loss, survival being abbreviated; the non-selected parental generation was more sensitive to desiccation than generations F₁ and F₂, both for healthy mollusks and those exposed to *S. mansoni* larvae; healthy

Table 6
Biomphalaria tenagophila. F₁, F₂ and non-selected parental generations. Observations of group II, after returning mollusks to water

Generation	Survival rates %	Cercariae elimination %	Elimination after desiccation (days)	Elimination period (days)
F ₁	35	35	2 (20%)	10 to 14
			4 (5%)	08
			6 (5%)	25
			18 (5%)	08
F ₂	50	50	24 (10%)	09
			22 (10%)	10
			2 (20%)	13
			2 (10%)	02
parental	20	10	3 to 4	30 to 32

Table 7
Biomphalaria tenagophila. F₁, F₂ and non-selected parental generations. Observations for groups I, III, IV and V after returning mollusks to the water

Group	Generation	Survival rates %	Cercariae elimination %	Cercariae elimination after desiccation (days)	Parasite's cycle (days)	Elimination period (days)
I	F ₁	67.85	10.71	20	49	19 (3.57%) 22 (7.14%)
	F ₂	45.0	30.0	32 (5%) 22 (5%) 20 (20%)	61 50 49	11 25 25 (15%) 60 (5%)
	parental	11.76	5.88	17 to 20	46 to 49	03 to 15
III	F ₁	68.4	10.5	8 (2.6%) 15 (2.6%) 27 (2.6%)	36 43 55	31 25 22
	parental	37.5	12.5 10.5	15 (2.6%) 46 (4.2%) 17 (8.3%)	43 74 45	34 64 59 (4.1%)
						63 (4.1%)
IV	F ₁	65.0	10.0	20	48	41 (5.0%) 27 (5.0%)
	F ₂	55.0	10.0	20	48	58 (5.0%) 65 (5.0%)
	parental	42.5	5.0	15	43	44 to 46
V	F ₁	75.0	20.0	22	50	43 (10.0%) 45 (5.0%) 99 (5.0%)
	F ₂	53.85	19.23	20 (11.55%) 27 (3.84%) 33 (3.84%) 24 (2.22%)	48 55 61 52	60 35 60 51
	parental	37.78	6.67	21 (4.45%)	49	08 to 23

Table 8

Cycle maintained by the SJ *Schistosoma mansoni* strain in *Biomphalaria tenagophila*, from F₁, F₂ and non-selected parental generations. Values in percentage (%)

Generation	<i>Schistosoma mansoni</i> cycle in <i>Biomphalaria tenagophila</i>													
	29 days	30 days	35 days	37 days	39 days	42 days	43 days	44 days	48 days	49 days	50 days	55 days	58 days	81 days
F ₁	0	0	3.5	6.3	0	0.7	4.2	7.7	1.4	0.7	2.9	2.9	2.8	0.7
F ₂	1.62	14.7	0	14.7	0	0	0	0	4.9	0	0	0	0	0
parental	0	3.8	2.9	0.9	11.4	12.4	0	0	0	0	0	0	0	0

mollusks were more resistant towards desiccation than those exposed to *S. mansoni* larvae; desiccation did not interrupt the development of *S. mansoni* larvae in mollusks from groups I through V, causing only a delay in the elimination of cercariae; the susceptibility of *B. tenagophila* to the SJ strain of *S. mansoni*, in mollusks kept in water during larvae incubation period, was similar for three generations.

RESUMO

Biomphalaria tenagophila (Orbigny, 1835) (Mollusca): adaptação à dessecação e suscetibilidade à infecção pelo *Schistosoma mansoni* Sambon, 1907

Foram realizados experimentos para testar a suscetibilidade de *B. tenagophila* à infecção pela cepa SJ do *S. mansoni*, das gerações F₁, F₂ e parental não selecionada. O potencial de adaptação de *B. tenagophila* à dessecação, em moluscos sadios e expostos às larvas do *S. mansoni* também foi estudado. A presença de muco e terra na abertura da concha protege os moluscos da dessecação, favorecendo a sobrevivência. Os moluscos sadios tiveram mais tentativas de proteção contra a dessecação do que os moluscos expostos às larvas do parasita. A mortalidade, durante a dessecação, foi maior entre os moluscos que permaneceram enterrados e apresentaram a abertura da concha desobstruída. O estágio de desenvolvimento das larvas do parasita influenciou na perda de peso e na sobrevivência dos moluscos. Quanto maior o período de dessecação, maior a perda de peso, sendo a sobrevida abreviada. A geração parental não selecionada mostrou-se mais sensível à dessecação que as gerações F₁ e F₂, tanto nos moluscos sadios como naqueles expostos às larvas do parasita. Os moluscos sadios foram mais resistentes à dessecação que os moluscos expostos às larvas do parasita. A dessecação não interrompeu o desenvolvimento de larvas do *S. mansoni* nos moluscos, causando apenas um atraso na eliminação de cercárias.

ACKNOWLEDGEMENTS

To FAPESP for financial support (FAPESP no. 95/6688-4).

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