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COMPETITIVE INTERACTION BETWEEN TWO SNAIL HOSTS OF SCHISTOSOMA MANSONI: LABORATORY STUDIES ON BIOMPHALARIA GLABRATA AND B. STRAMINEA

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SUMMARY

Under laboratory conditions, competition has been observed between **Biomphalaria glabrata** and **B. straminea**, the snail intermediate hosts of **Schistosoma mansoni** in Northeastern Brazil. When the two species occupy the same habitat, **B. straminea** appears to be the dominant species and, under certain circumstances, may replace **B. glabrata**. Competition appears to be exerted, in part, as a consequence of inter-specific crowding which reduces the fecundity of **B. glabrata**. When this reduced fecundity is coupled with the greater hatch-rate of **B. straminea** eggs, then the net effect is a significant increase in the **B. straminea** population at the expense of **B. glabrata**. **B. straminea** appears to be more aggressive than **B. glabrata** and readily invades the territory of the latter species. No evidence for either food competition or for the production of inhibitory substances by **B. straminea** was elicited by the present study.

INTRODUCTION

Although competitive exclusion, as a means of biological control, has been assiduously pursued by entomologists, it has received scant attention from those interested in the control of the snail hosts of schistosomiasis. Circumstantial evidence as well as some preliminary studies suggest that the phenomenon may, in fact, be operational in nature with regard to fresh-water snails and the method appears to warrant further investigation. For example, the absence of **Biomphalaria glabrata** from the Greater Antilles, except Puerto Rico, where species of **Helisoma** are the dominant planorbid snails, may represent an example of competitive exclusion. Moreover, in Puerto Rico, where species of **Helisoma** and **B. glabrata** both occur, they rarely inhabit the same body of water[9,12]. In addition, some recent studies[1,2,6,7,8,13] suggest that the presence of **Helisoma** may inhibit reproduction and reduce population densities in several species of schistosome intermediate hosts. WRIGHT[14] has hypothesized that the geographic distribution of the three schistosome snail hosts found in Brazil (**B. glabrata, B. straminea, B. tenagophila**) may be attributed, in part, to competitive exclusion. In this regard, it has been noted by PARAENSE[11] that the introduction of **B. glabrata** from Aracaju into a habitat of **B. tenagophila** in the state of Guanabara resulted in the displacement of the latter species. Likewise, the introduction of **B. tenagophila** from São Paulo into a **B. glabrata** habitat in Belo Horizonte caused the displacement of the autochthonous population. BARBOSA[4] reported on the natural displacement of a population of **B. glabrata** in Pernambuco after **B. straminea** was somehow introduced into its habitat and stated that, "The results of the study suggest a competitive displacement of **Biomphalaria glabrata** by **B. straminea**." The intent of the present study

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is to further explore the suggestive observations of Barbosa and to determine by laboratory models if, in fact, displacement occurs and what factors may be operating to the advantage of **B. straminea**.

MATERIALS AND METHODS

Two albino strains of **B. glabrata**, one from Belo Horizonte, Brazil (BH) and the other a hybrid strain (PR/B) originally derived from a cross between a Puerto Rican and a Brazilian snail, were used in the study. The strain of **B. straminea**, used as a competitor, was derived from snails collected in Recife, Brazil. All experiments were conducted in a temperature controlled room (25 ± 1.°C) and the snails exposed to ceiling level fluorescent lights cycled for 10 hr of light and 14 hr of darkness. Unless specified, fresh Romaine lettuce was supplied in known amounts at selected intervals, details being given in the description of each experiment. At least one and usually several replicates were performed for each experiment.

Competition experiments

The first experiment consisted of 3 aquaria, each containing 3 liters of water and stocked so that one had 10 **B. glabrata** (6-9 mm diam.), another 10 **B. straminea** (6-7 mm diam.), and the third a mixed population of 5 snails of each species. The aquaria were aerated and food was added twice a week so that the total amount per week equaled 20 gm for the first 9 weeks, 30 gm for the next 9 weeks, and 40 gm per week thereafter. All snails in each aquaria were counted at 30 day intervals and the experiment ran for 180 days.

A second experiment was designed to test the effect of varying the size of the initial **B. straminea** population. Thus, 4 aquaria containing 5 liters of water were set up as follows: 5 **B. glabrata** (PR/B, 7-9 mm) and 5 **B. straminea** (3-6 mm); 5 **B. glabrata** and 15 **B. straminea**; 5 **B. glabrata** and 50 **B. straminea**, and one with no **B. straminea**. The experiment ran for 20 weeks and all snails in each aquaria were counted at 4 to 5 week intervals. Food was supplied as described in the first experiment.

A third experiment was designed to test the effect of additional selection pressure by exposing a mixed population to miracidia of **Schistosoma mansoni**. Miracidia obtained from the livers of mice infected with a Puerto Rican strain of **S. mansoni** were introduced weekly, at a rate of 100 miracidia per snail, into a 3 liter aquarium containing 15 (5-7 mm) snails of each species. Snails were counted at 4 week intervals. Preliminary studies indicated that the PR/B strain was highly susceptible to infection with the strain of **S. mansoni** employed, whereas **B. straminea** was refractory.

Crowding phenomenon

Although intra-specific crowding, with a concomitant reduction in growth and reproduction, has been repeatedly demonstrated in populations of fresh-water snails, there is, to our knowledge, no published evidence of the phenomenon occurring in mixed species populations. An experiment was designed, therefore, to determine if one species could exert a crowding effect on a closely related species and to what extent. Five aquaria, each containing 4 liters of water, were stocked with snails so that there was a mixed population aquarium containing 15 **B. glabrata** (PR/B) and 14 **B. straminea** and two control aquaria for each species, one with 15 snails (uncrowded) and the other with 30 (crowded). The initial mean size of all snail populations was 4.4 mm (range 3.6 — 5.5 mm). Aquaria were drained weekly, egg masses removed, and the individual eggs counted. Snails were counted and measured at 2 week intervals and food was supplied in excess at weekly intervals.

Growth and reproductive potential

Two aquaria, each containing 1.8 liters of water, were stocked with 20 young snails of each of the appropriate species and an effort was made to compare their early rate of growth and their reproductive potential. The snails were approximately 30 ± 7 days of age at the start of the experiment and had a mean size of 2.8 mm for the **B. straminea** and 3.4 mm for the BH strain of **B. glabrata.** At weekly intervals the snails were measured, eggs masses removed and the eggs counted, and the egg masses then placed in separate containers for up to 2 weeks so that the percentage of hatch could be determined.

Territorial exclusion

The following experiment was designed to determine if the presence of one species would exclude the migration of the other into its realm. An apparatus (Fig. 1), consisting of two chambers joined by a small bridge, was constructed from plastic and when filled contained 610 ml of water. Base-line values were established for each of the species by introducing a group of 10 snails (7-8 mm) into one of the chambers and allowing them, after a 5 min conditioning period, to freely migrate for one hour. Seven groups from each species were tested and the data pooled. Once base-line values were established, groups of the two species were placed simultaneously into opposite chambers and allowed to migrate for one hour. Data from 7 trials were pooled, and then compared to base-line results by means of a Chi Square Test.

Food consumption and assimilation

Five snails (7-9 mm) of each species were placed as individual groups into special plastic

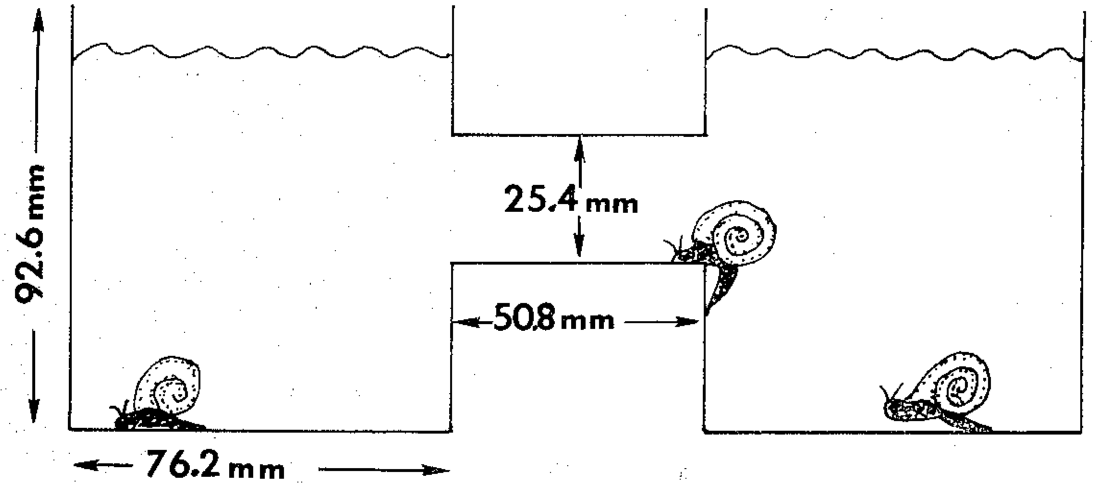

Fig. 1 — Diagrammatic sketch of apparatus used to test for territorial exclusion and vagility.

containers (Fig. 2) and held for 48 hr without food. The containers were then emptied of water and feces, refilled with water, and a known amount of dried lettuce added as food. At the end of 24 hr, the uneaten lettuce and the feces were collected, dried in an incubator over Drierite (R) for 48 hr, and then weighed on an electronic balance. Simple mathematical manipulation permitted a rough estimate of the amount of lettuce consumed as well as the portion actually incorporated into the snail. Data from 16 trials employing different groups of snails were pooled for each species for statistical analysis.

Growth inhibitory substances

It has been suggested by ABDALLAH & NASR [1] that **Helisoma duryi** successfully competes with species of **Biomphalaria** and **Bulinus** by secreting a substance into the water which inhibits the development of the eggs of these species. To test for the possible excretion of an inhibitory substance produced by **B. straminea**, newly deposited egg-masses of **B. glabrata** were placed in water obtained from an aquarium in which several hundred **B. straminea** had been maintained for more than one month. The egg-masses were examined daily for up to 14 days to determine the development and rate of hatching. Egg-masses were also placed in water from an aquarium containing several hundred **B. glabrata** and served as a control. A second series of experiments was conducted as follows: Five one-liter beakers were filled with distilled water and **B. straminea** were added so that the beakers contained 20, 10, 5, 3 and 1 snail/s respectively. A beaker containing 20 **B. glabrata** was used as a control. After a 2 day conditioning period, egg-masses of **B. glabrata** were added to each beaker and allowed to develop. In one series of experiments, the beakers were continually aerated, in another there was no aeration.

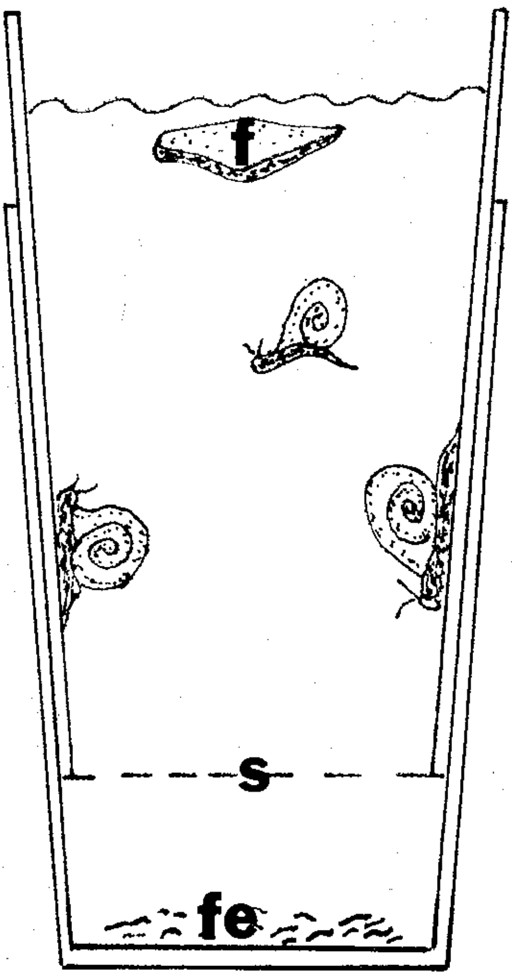

Fig. 2 — Diagrammatic sketch of apparatus used in food consumption and assimilations studies. The apparatus consisted of two chambers separated by a screen (s). Food (f) was placed in the upper chamber with a group of snails. At the end of 24 hr uneaten food was removed from the upper chamber and feces (fe) from the lower.

RESULTS

Data from the competition experiments demonstrate that **B. straminea** effectively competes with **B. glabrata** and under some circumstances may replace the latter species. Moreover, the speed at which displacement occurs is largely dependent upon the initial frequencies of the competitor and target species in the overall population. Thus, in the first experiment, where both target and competitor are of equal frequency, complete replacement occurred between 5 and 6 months (Table I). When the frequency of the competitor increased, replacement became more rapid (Exp. 2, Fig. 3) and was accomplished after 3 months or less. The addition of increased environmental pressure, supplied by the introduction of miracidia and the subsequent infection of the susceptible target population, reduced replacement time by as much as 50 per cent (Table II).

TABLE I

Changes in the frequency of competitor (**B. straminea**) and target (**B. glabrata**) snails in a mixed population in which the proportion of each species was initially equal. Exp. 1.

Snail Species	Frequency on Day 0	30	60	90	120	150	180
B. glabrata (BH)	0.5	0.102	0.137	0.016	0.24	0.01	0
B. straminea	0.5	0.898	0.863	0.984	0.76	0.99	1.00

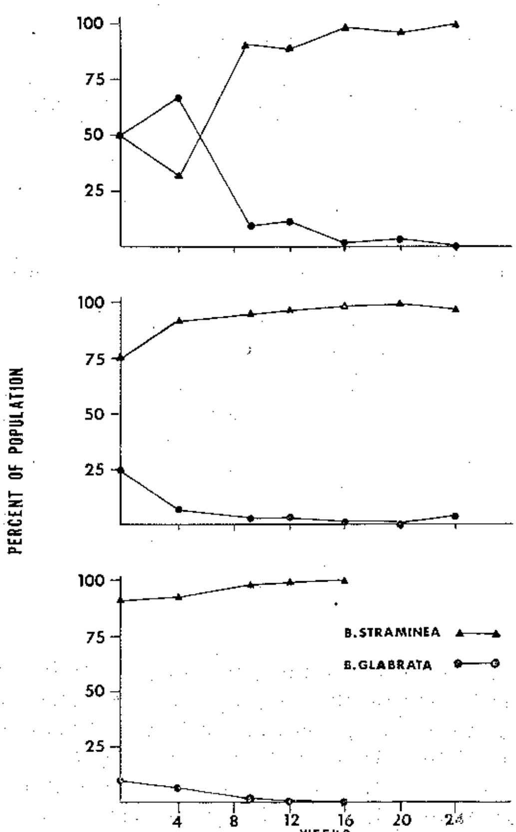

Fig. 3 — Results of competition experiments in which the intitial frequency of each species was varied. In the upper graph, the intitial frequency was equal. In the meddle graph, the ratio of **B. straminea** to **B. glabrata** was 3:1. In the bottom graph, the frequency of **B. straminea** to **B. glabrata** was approximately 10:1.

TABLE II

Changes in the frequency of competitor (**B. straminea**) and target (**B. glabrata**) snails in a mixed population in which 100 miracidia per snail were added weekly. Initially each of the species was equal in number.

Snail Species	Frequency on Day 0	30	60	90	120
B. glabrata (BH)	0.5	0.136	0.085	0.023	0
B. straminea	0.5	0.864	0.915	0.977	1

Our observations further suggest that in mixed species populations the crowding phenomena is operating, and that each species appears to inhibit, to some degree, the fecundity and growth of the other (Table III, Fig. 4). Data presented in Table III demonstrate that for each species, the number of eggs laid, the eggs per mass, and the eggs/snail/day were less in the mixed population than in uncrowded control populations. In the mixed population, **B. straminea** laid more eggs (11,249) than did **B. glabrata** (6,950) and the number of eggs per snail was significantly greater ($0.02 < P < 0.05$) when analyzed by the t-test for paired data. Differences in size were also apparent between snails of the mixed population and those in uncrowded populations (Fig. 4).

TABLE III

Effect of inter- and intra-specific crowding on the fecundity of **B. glabrata** and **B. straminea**

Species	Time (Wk)	Uncrowded (15) No. eggs	Mean eggs/mass	Eggs/snail/ day	Crowded (30) No. eggs.	Mean eggs/mass	Eggs/snail/ day	Mixed Population (15+) No. eggs	Mean eggs/mass	Eggs/snail/day day
	1	—	—	—	—	—	—	—	—	—
	2	26	2.0	.2	7	7.0	<.1	—	—	—
	3	510	17.0	4.9	328	13.1	3.1	12	12.0	.1
B. glabrata	4	1145	17.9	10.9	1755	16.0	8.4	115	8.8	1.1
	5	2033	25.7	19.4	2474	21.5	11.8	989	20.2	9.4
	6	2574	25.2	24.5	3075	20.4	14.6	1536	22.9	14.6
	7	3828	27.7	36.5	4154	25.0	19.8	2076	25.0	19.8
	8	3955	34.1	37.7	3382	28.4	16.1	2222	28.9	21.2
	1	—	—	—	—	—	—	—	—	—
	2	566	14.9	5.4	733	12.0	3.5	505	16.3	4.8
	3	668	19.1	6.4	1131	16.6	5.4	698	15.5	6.6
B. straminea	4	1895	23.4	18.0	2182	18.2	10.4	1245	18.6	11.9
	5	2996	24.0	28.5	3808	19.8	18.1	1875	22.0	17.9
	6	3538	29.0	33.7	4296	19.9	20.5	2230	23.5	21.2
	7	3596	30.7	34.2	5586	24.1	26.6	2390	25.7	22.8
	8	3613	34.4	34.4	5757	23.4	27.4	2306	25.6	22.0

Results of studies on early growth and hatch rate of the two species were of considerable interest. During the 17 weeks that the populations were followed, increments in the mean shell size varied from 0.4 ± 0.3 mm/wk for **B. straminea** and 0.6 ± 1.0 mm/wk for **B. glabrata**. The mean overall size of the two populations went from 2.8 mm to 9.5 mm and from 3.4 mm to 13.4 mm for **B. straminea** and **B. glabrata** respectively. Reproduction first occurred in **B. straminea** when the population had a mean size of 5.9 ± 0.6 mm and in **B. glabrata** at a mean of 8.0 ± 1.00 mm. This corresponds to ages of 65 ± 7 days and 72 ± 7 days respectively. The percent of hatch of the eggs of the two species was followed for 12 wk, and it was observed that **B. straminea** had a mean hatch rate of 70.5% and that of **B. glabrata** 60.4%. When weekly results were compared by a T-test for paired data, the difference in the hatch rate of the two species proved to be significant ($0.02 < P < 0.05$). Thus, even though **B. glabrata** produced more eggs (11,926) than did **B. straminea** (11,031) during

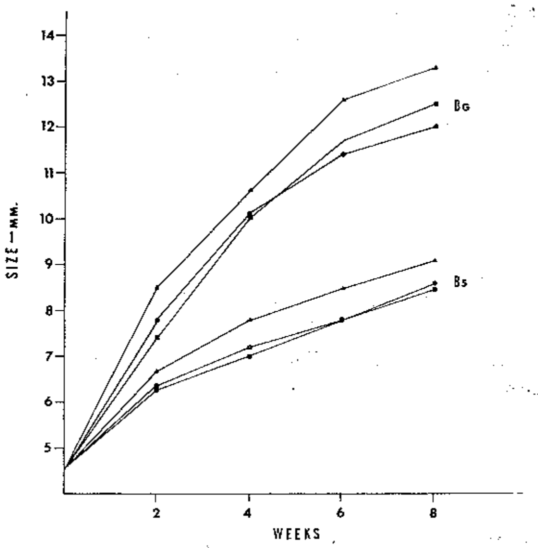

Fig. 4 — The effects of crowding on the growth of **B. glabrata** and **B. straminea** in homogenous and mixed populations. The upper series of lines (Bg) represents populations of **B. glabrata**, the lower (Bs) **B. straminea**. Squares represent mixed populations of the two species, circles the crowded controls, and triangles the uncrowded controls.

the observation period, **B. straminea** exhibited a potential net increase of 574 new snails in excess of **B. glabrata**. If we interpolate the data of the mixed population aquaria from the crowding experiments in the light of the hatch rates, the advantage to **B. straminea** becomes even more dramatic — a potential net increase of over 3,700 new snails.

Our experiments gave no indication of territorial exclusion by one species or the other. However, **B. straminea** was found to invade the territory of **B. glabrata** more frequently than the latter invaded its territory. When the data (Table IV) are analyzed by the Chi Square test, employing 1 degree of freedom, the difference in invasion was significant. It should be noted, however, that the difference between the activity of **B. straminea** in the presence of **B. glabrata** was not significantly different from its behavior when placed in the system by itself. It appears that **B. straminea** is a more vagile species than is **B. glabrata**.

Under our experimental conditions no significant differences were noted in either the food consumption or assimilation of food by either species. The mean food consumption for **B. straminea** was 35.9 ± 8.1 mg lettuce/day/snail and that of **B. glabrata** was 32.5 ± 9.3 mg lettuce/day/snail. The amount of food estimated to be actually assimilated per snail was 5.5 mg for the former species and 5.2 mg for the latter.

TABLE IV

Summary of territorial exclusion experiments. Seven trials with ten snails of each group.

Group	Remained in Orig. Chamber	Moved to New Chamber	Total Snails Tested
B.g alone (control)	64	6	70
B.g. to **B.s.**	61	9	70
B.s. alone (control)	55	15	70
B.s. to **B.g.**	51	19	70

B.g. to **B.s.** vs. **B.g.** alone .... $X^2 = 0.0672$ not significant
B.s. to **B.g.** vs. **B.s.** alone .... $X^2 = 0.6217$ not significant
B.g. to **B.s.** vs. **B.s.** to **B.g.** .. $X^2 = 4.4642$ significant ($0.02<P<0.05$)
B.s. alone vs. **B.g.** alone ...... $X^2 = 4.5378$ significant ($0.02<P<0.05$)

DISCUSSION

Biomphalaria glabrata and **B. straminea** serve as the intermediate hosts of **S. mansoni** in northeastern Brazil, and despite their overlapping ranges rarely, if ever, occur in the same habitat [5]. In fact, we are aware of only two published accounts in which the two species have been reported as cohabitants of the same biotope. The first, in Corrego do Bambu, a small stream at Governador Valadares, Minas Gerais [10], and the second, a small stream near Recife, Pernambuco [4]. In the latter case, **B. glabrata** was replaced by **B. straminea** after a period of 3 years.

B. glabrata generallly inhabits permanent water bodies in the littoral and forest regions of northeastern Brazil, although some populations may also colonize temporary ponds in the drier regions [3]. **B. straminea** is the dominant species in the more arid zones, but occurs throughout the entire region. Ecological requirements appear, therefore, to act to some extent as a barrier to dispersal and intermixing of the species. It has been suggested by WRIGHT [14] and BARBOSA [4] that the present distribution of the two species may be the consequence of inter-specific competition.

Our experiments support the thesis that the two species are in fact competitors and that **B. straminea**, under certain circumstances, may replace **B. glabrata**. It would appear that competition is exerted, in part, as a consequence of inter-specific crowding which reduces the fecundity of **B. glabrata**. When this reduced fecundity is coupled with the greater hatch-rate observed in **B. straminea**, then the net result is a significant increase in the **B. straminea** population at the expense of the **B. glabrata**. On occasion, we have observed efforts by **B. straminea** to inseminate **B. glabrata**; however, we have not been able to confirm Barbosa's observation [4] on hybridization. A second factor, which may contribute to the competitive superiority of **B. straminea** is its vagility and aggressiveness in invading territory occupied by **B. glabrata**. In this regard, our findings in the laboratory are in agreement with the field observations of BARBOSA [4]. Our results provide no evidence of food competition; however, it must be noted that experiments utilizing lettuce are highly artificial and that under natural conditions algae and other elements of the micro-fauna and flora probably serve as a food source. Likewise, we have no evidence of the production of inhibitory substances by **B. straminea** which might contribute to its effectiveness in replacing **B. glabrata**.

We do not wish to imply that our observations in the laboratory are direct reflections of what occurs in nature; however, we do feel that they provide insight into mechanisms which might account for the competitive interaction observed between these two host species.

RESUMO

Interação competitiva entre dois moluscos hospedeiros do Schistosoma mansoni: **estudos de laboratório sobre** Biomphalaria glabrata **e** B. straminea.

Em condições de laboratório observou-se a ocorrência de competição entre **Biomphalaria glabrata** e **B. straminea**, hospedeiras intermediárias do **Schistosoma mansoni** no Nordeste do Brasil. Quando as duas espécies ocupam o mesmo habitat, **B. straminea** parece ser a espécie dominante e, em certas condições, pode substituir **B. glabrata**. A competição parece exercer-se, em parte, através da redução da fecundidade de **B. glabrata** por efeito de massa (crowding effect) interespecífico. Quando esta redução de fecundidade combina-se com maior taxa de eclosão dos ovos de **B. straminea**, o resultado é um aumento significativo da população de **B. straminea** à custa de **B. glabrata**. **B. straminea** parece ser mais agressiva que **B. glabrata**, invadindo prontamente o território desta última. O presente estudo não permite concluir pela existência de competição alimentar nem pela produção de substâncias inibidoras da parte de **B. straminea**.

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REFERENCES

1. ABDALLAH, A. & NASR, T. — **Helisoma H. duryi** as a means of biological control of schistosomiasis vector snails. **J. Egyptian Med. Assoc. 56**: 514-520, 1973.

2. AYAD, N.; MOUSA, A. H.; ISHAK, M. M.; YOUSIF, F. & ZAGHLOUL, S. — A preliminary study on biological control of the snail intermediate hosts in U.A.R. by **Helisoma duryi** snails. **Hydrobiologia 35**: 196-202, 1970.

3. BARBOSA, F. S. — Aspects of the ecology of the intermediate hosts of **Schistosoma mansoni** interfering with the transmission of bilharziasis in north-eastern Brazil. In: Bilharziasis, pp. 23-35. Ed. G. E. W. Wolstenholm & M. O'Connor. Boston, Little, Brown & Co., 1962.

4. BARBOSA, F. S. — Possible competitive displacement and evidence of hybridization between two Brazilian species of planorbid snails. **Malacologia 14**: 401-408, 1973.

5. BARBOSA, F. S. & OLIVIER, L. — Studies on the snail vectors of bilharziasis mansoni in north-eastern Brazil. **Bull.** W.H.O. **18**: 895-908, 1958.

6. EL-HASSAN, A. A. A. — **Helisoma tenue** and **Physa acuta** as biological means of control against **Bulinus trun-**

catus and **Biomphalaria alexandrina**, snail intermediate hosts of schistosomiasis in Egypt. **Proc. Third Internat. Congr. Parasitol.** (Munich) 3: 1597-1598, 1974.

7. FRANDSEN, F. — The suppression, by **Helisoma duryi** of the cercarial production of **Schistosoma mansoni**-infected **Biomphalaria pfeifferi. Bull. W. H. O. 53:** 385-390, 1976.

8. FRANDSEN, F. & CHRISTENSEN, N. ϕ. — Effect of **Helisoma duryi** on the survival, growth, and cercarial production of **Schistosoma mansoni**-infected **Biomphalaria glabrata. Bull. W.H.O. 55:** 577-580, 1977.

9. HARRY, H. W. & HUBENDICK, B. — The freshwater pulmonate mollusca of Puerto Rico. **Meddelanden Göteborgs Musei Zool. Avdelning 136:** 3-77, 1964.

10. PARAENSE, W. L. — Studies on **Australorbis centimetralis.** II — Biospecific characterization. III — Generic status. **Rev. Brasil. Biol. 15:** 341-348, 1955.

11. PARAENSE, W. L. — Planorbídeos hospedeiros intermediários do **Schistosoma mansoni.** In: Esquistossomose mansoni, pp. 13-30. Ed. A. S. Cunha. São Paulo, Sarvier Edit. Livros Médicos Ltda. & Edit. Univ. São Paulo, 1970.

12. PIMENTEL, D. & WHITE JR., P. C. — Biological environment and habits of **Australorbis glabratus. Ecology 40:** 541-550, 1959.

13. RASMUSSEN, O. — Biological control of **Biomphalaria pfeifferi** by **Helisoma duryi. Proc. Third Internat. Congr. Parasitol.** (Munich) 3: 1598-1599, 1974.

14. WRIGHT, W. H. — Geographical distribution of schistosomes and their intermediate hosts. In: Epidemiology and Control of Schistosomiasis (Bilharziasis), pp. 32-249. Ed. N. Ansari. Basel, S. Karger, 1973.

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