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WHY IT IS DIFFICULT TO CONTROL *BIOMPHALARIA GLABRATA,* THE VECTOR SNAIL OF SCHISTOSOMIASIS

PEDRO JURBERG

Instituto Oswaldo Cruz, Departamento de Biologia, Laboratório de Comportamento Animal, Caixa Postal 926,
20001 Rio de Janeiro, RJ, Brasil

The author studied some protective behaviors of Biomphalaria glabrata *(vertical movements, response to molluscicides and response to water currents) and demonstrated how these activities hamper the control of this snail, which is the main vector of schistosomiasis in Brazil.*

In Brazil, schistosomiasis is caused by *Schistosoma mansoni* (Sanbon), with fresh-water pulmonate gastropod mollusks acting as intermediate hosts. The three species found infected in nature are *Biomphalaria glabrata, B. straminea* and *B. tenagophila.* Two other species *(B. amazonica* and *B. peregrina)* could be infected in the laboratory (Paraense, 1972), thus becoming potential vectors of *S. mansoni.* Of the three species, *B. glabrata* is the most important because of its wider geographic distribution and its occurrence is almost always associated with schistosomiasis (Paraense, 1972).

The transmission of schistosomiasis can be controlled by reducing the populations of vector snails (Katz, 1986). This reduction can be achieved by three approaches, i. e., the use of molluscicides, biological control and environmental control (WHO, 1965; Thomas, 1973; McCullough, 1986). Even though these methods are effective, some snails succeed in surviving and repopulate treated or manipulated breeding sites. This may be due to two reasons: 1) the reproductive characteristics of the species, which is a hermaphrodite capable of self-fecundation (Brumpt, 1941) and very prolific. According to Paraense (1955), a single specimen may give origin to more than 10 million snails within 4 months; 2) some protective behaviors used by the snail.

We call protective behaviors activities which, due to their occurrence induced by natural causes such as rain, changes in temperature, genetic patrimony, or by artificial causes such as response to molluscicides or to sanitary engineering measures, may favor the survival of some snails, which will repopulate breeding sites within a short period of time as soon as conditions become ideal again.

The study of planorbid behavior has been recommended by the World Health Organization (WHO, 1965), bu few investigations have been made and unfortunately their conclusions have not been taken into account in the planning of control campaigns or in test of new molluscicides.

Studies on the biology, ecology and physiology of snails (for reviews see Andrade, 1959; Paraense, 1972; Appleton, 1978) have provided detailed data on their habits and causal relationships, but the etologic approach used by us differs from these classical studies in that it makes an attempt to record and describe all stages occurring between cause and effect in order to determine the adaptative advantages of behavior in relation to survival. The importance of the behavior of these snails with respect to survival has been reviewed by Pieri & Jurberg (1981a), who examined the following behavioral patterns presumably related to protection: retraction of the cephalopodal mass into the shell, leaving dried out sites, leaving the water, moving away from toxic agents, and burrowing. In that study, it was observed that little was written on the triggering mechanisms and the adaptative values of such behaviors in relation to survival.

The objective of the present paper is to describe some aspects of the behavior of *B. glabrata* and to demonstrate how this part of the snail's biology may be influencing the survival of some individuals, thus facilitating the later repopulation of treated breeding sites.

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Vertical movements of B. glabrata

The possibility of *B. glabrata* moving to deep regions and surviving there even temporarily may favor the survival of some snails in deep breeding sites treated with the usual molluscicides, since the latter are applied to the surface, which is considered the most frequent habitat (Paraense & Santos, 1953; Andrade, 1959; Freitas, 1976; Appleton, 1978).

Deshiens & Jadin (1954) demonstrated that specimens of *B. glabrata* and *B. adowensis* survived for 24 days at 10 m depths in boxes which were lifted to the surface every 8 days to feed the snails. Gillet et al. (1960), in similar experiments, noted that *B. pfeifferi* was able to survive 31 days at 15.5 m when immersed in boxes that were lifted to the surface twice a week for feeding. Jurberg et al. (1987) published a review of planorbids living in deep sites.

To test the possibility that *B. glabrata* moves actively to deep sites, we built 3 columns of transparent plastic respectively measuring 1, 8 and 10.40 m in height where we investigated the voluntary descending and climbing up behavior, as well as copulation, ovipositing, feeding, and defecation. We found that the snails descended and climbed up voluntarily and carried out all of the activities listed above even at the bottom of the 10.40 m column (Jurberg et al., 1987).

To test whether *B. glabrata* could withstand greater depths, we built transparent chambers capable of withstanding pressures corresponding to 50 m depths. Snails placed in them survived for 48 hours with no significant difference from the control group maintained at normal pressure (Jurberg et al., 1988c). We also noted that abrupt changes in pressure simulating the descent of snails from the surface to a depth of as much as 50 m and the return to the surface within 3 did not affect survival or later ovipositing, indicating that these animals could descend to this depth and climb up again without any apparent damage (unpublished data).

Using the same chambers, we found that *B. glabrata* can survive up to 8 days at pressures corresponding to 50 meters with no apparent deleterious effect attributable to pressure, since there was no significant difference between the experimental and the control groups. (unpublished data).

Considering that descending to a depth of 10.4 m and surviving at pressures corresponding to 50 m would be of little usefulness if the snails cannot spend a long time without returning to the surface, we tested the animals in aquaria that did not permit them to return to the surface and noted that the snails were able to survive for 92 days without performing gas exchange at the surface (Jurberg et al., 1982).

These data indicate that the usual molluscicides employed to reach snails staying close to the surface may be ineffective when the animals are at the bottom of water reservoirs or lakes at the time of treatment.

The results obtained in the laboratory confirm the data obtained for planorbids at great depths. These animals, even though they are pulmonated and preferentially use atmospheric oxygen, can survive at greater depths since they can stay in anaerobiosis for 16 hours, as observed by Von Brandt et al. (1948) for *B. glabrata* and *B. pfeifferi*, so that a decrease in oxygen in deep places does not prevent survival. Another factor which favors survival is that oxygen rates compatible with survival can be found even in deep sites, such as the Don Helvecion lake in the Rio Doce valley, where 3.36 mg/l oxygen are found at 22.2 meters (Mitamura & Hino, 1986).

Response of planorbids to molluscicides

When a molluscicide is used to control a snail population, the dose applied is calculated in such a way as to kill the target population with a certain margin of safety. However, the possibility should be taken into account that the usual molluscicides, when they dissolve, will reach certain regions of the breeding site at sublethal concentrations. If these substances act as repellents, they may cause part of the population to leave the water, with consequent later repopulation of the site after conditions return to normal for the snails.

The literature concerning the behaviors of *B. glabrata* in relation to molluscicides is limited, as shown by Pieri & Jurberg (1981 a, b; Jurberg et al., 1988a), and the results reported are qualitative. Nolam et al. (1953) found that snails left the water in the presence of many phenol compounds tested by them. Etges (1963) observed the ability of *B. glabrata* to avoid high doses when a concentration gradient of copper, barium, cobalt and zinc salts and sodium pentachlorophenate is used. Etges & Gilbertson (1966) obtained similar results with sodium pentachlorophenate, copper sulfate and DINEX. Souza & Paulini (1967), in a study of the mortality curve for *B. glabrata* at different doses of sodium pentachlorophenate, observed that the snail was able to avoid contact with the molluscicide when the cover used to prevent its exit was removed. When the remaining conditions were kept constant, these apparently small modifications caused a 3-fold increase in the LD_{50} rate.

In a detailed study of the behavior of *B glabrata* in relation to sublethal doses of copper sulfate, Pieri & Jurberg (1981b) observed that the frequency of a series of behavioral parameters (time to the first ascent to the surface, frequency of exists from the water, time of permanence in the water) varied as a function of the dose of the product. In that study, the authors showed that the use of behavioral parameters as indicators of the toxic action of products is fully viable and that this procedure permits the detection of the toxic effect of a substance at concentrations much lower than those used in conventional lethality tests.

Specific tests for the investigation of behavioral patterns of planorbids that favor survival or make the animals more vulnerable to molluscicides are recommended by the World Health Organization (WHO, 1965) to complement lethality assays. However, in the tests proposed by the WHO itself to determine the lethality of a product (calculation of LD_{50} or LD_{90}), the snails are exposed ot increasing doses of the product inside flasks in which they are prevented from reaching the surface by an obstacle (a net, for example). A linear dose/lethality relationship is generally determined, but without considering the possibility that the animal will exhibit behaviors related to getting out of the solution either as part of its own behavioral repertory or as a function of the repellent effect of the substance tested.

In our test of the molluscicidal activity of *Euphorbia tirucalli*, therefore, we performed the biological assays in such a way as to permit the determination of an index denoted "exit index", which corresponds to the percentage of animals found outside the solution at the time of completion of the exposure time to the different concentrations of molluscicide. This index provides a level of repellence response in the evaluation of the molluscicide, and is easy to execute, using the same protocol as proposed by the WHO but not the obstacle that prevents the snails from leaving the solution. The exit index obtained for *E. tirucalli* was 7.6% for the experimental groups as a whole and 3.2% for the control (Jurberg et al., 1985).

On the basis of the studies cited above, we later standardized a technique to determine whether behavior effectively contributes to the survival of *B. glabrata* specimens exposed to molluscicides. We utilized *Phytolacca dodecandra*, which is one of the most promising molluscicides of plant origin (Kloos & McCullough, 1982; Hostettmann, 1984). The tests were carried out in aquaria with individual interconnected compartments fitted with side edges that simulated the margins of a body of water, so as to permit the animal to stay out of the solution when leaving it or to return to the liquid medium. The behavior of each animal during exposure to the molluscicide was recorded by the time-lapse cinematography technique, and surviving and dead specimens were identified at the end of the test. By analyzing the behavior of each animal and whether or not it survived we concluded that leaving the liquid medium and the time spent outside the solution significantly contributed to snail survival. The high exit index observed for the experimental groups as a whole (40% as opposed to 7% for the control) permitted us to conclude that this parameter is a good indicator of the occurrence of a behavioral pattern related to snail protection (Jurberg et al., 1988b).

The use of the exit index to determine the repellent action of molluscicides should be interpreted with caution, using certain specific criteria. First of all, we should consider a natural exit index, i. e. certain planorbid populations leave the medium naturally, at least under laboratory conditions. This natural exit index is provided by the control group in which the snails are under the same conditions as the snails to be tested, but without any molluscicide. The second criterion to be followed is that the exit index is valid only for the population which is being tested. Different populations (or species) will give different exit indices. Third, the numerical expression should be considered when the natural exit index (for the control group) is compared with the exit index obtained up to (but not including) the concentration corresponding to LD_{50}, since the exit index will decrease with increasing lethality.

Preliminary results obtained in tests carried out on *B. glabrata*, *B. tenagophila* and *B. straminea* maintained in the laboratory or newly-collected in the field using *P. dodecandra* confirmed the above considerations (unpublished data).

Role of behavior in B. glabrata *survival at sites treated with molluscicides – (Bayluscide)*

This study originated from observations of a rainwater draining ditch and of a natural spring (Manguinhos, RJ, Brazil), where *B. glabrata* specimens proved to be able to move vertically upstream on the walls of the ditch.

Horizontal upstream migration by *B. glabrata* has been reported by several investigators in field studies (Luttermoser & Castellanos, 1945; Pimentel et al., 1957; Scorza et al., 1961; Paulini, 1963; Etges & Frick, 1966; Bousfield, 1978, 1979). The last author also carried out experiments in the laboratory and concluded that rheotaxis was triggered by chemical substances (chemoattractants).

Our observations led to field and laboratory studies which permitted us to conclude that: a) this species is able to climb up vertical surfaces both in the field and in the laboratory; b) a water current as a physical stimulus is sufficient to trigger this behavior (rheotaxis), the only requirement being that the current should be moderate; c) there are signs that *B. glabrata* habituates with respect to rheotaxis after 24 hours; d) rheotaxis on vertical surfaces can facilitate population dispersal; e) the relationship between rheotaxis and habituation should be considered as a factor causing snail clustering in water pools and may contribute to snail location. (Jurberg et al., 1988a).

The climbing behavior in response to a water stream should be taken into account in campaigns of snail eradication, since it may contribute to snail protection. Indeed, a recent treatment of the ditch under study with Bayluscide eliminated the snails but did not affect those that had climbed up the wall and the draining pipes, which repopulated the treated sites within three months. We also observed in the field that *B. glabrata* responds to the water stream that flows down the walls of the ditch on rainy days by avoiding being carried away by the currents inside the ditch.

CONCLUSION

We had the opportunity of analyzing some protective behaviors that favor snail survival and may affect the repopulation of breeding sites even after they are treated with molluscicides. Even though all of these activities favor the snails, we believe that a larger number of investigations of behavior, ecology and new molluscicides should be carried out so that we may be able to reduce the transmission of schistosomiasis by controlling the vector snails.

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