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POTENTIAL SCHISTOSOME-VECTOR SNAILS AND ASSOCIATED TREMATODES IN RICEFIELDS OF CORRIENTS PROVINCE, ARGENTINA. PRELIMINARY RESULTS

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Considering the possibility of introduction of schistosomiasis mansoni into Argentina as a consequence of dam construction on the Rio De La Plata basin, preliminary studies have been carried out on agrosystems such as ricefields in Corrientes province with the following purposes: 1) to survey and estimate the relative abundance of planorbids and identify potential vector species; 2) to identify environmental factors capable of influencing Biomphalaria *population dynamics; and 3) to find out snail-parasite associations and estimate snail infection rates in order to detect possible competitive interactions between larval stages of native trematodes that could be used in biological control of* Schistosoma mansoni.

Three potential schistosome vectors were detected in ricefields, namely Biomphalaria straminea, B. tenagophila *and* B. peregrina, *although* B. orbignyi, *a species refractory to infection with* S. mansoni, *proved the most frequent and abundant.*

Positive correlations ($P < 0.05$) were found between Biomphalaria *abundance and some environmental parameters: conductivity, hardness, calcium, nitrites plus nitrates, ammonium and bicarbonates. Water temperature correlation was negative ($P < 0.05$). No correlation ($P > 0.05$) was found in total iron, phosphates (SRP), pH and soil granulometry.*

Echinocercariae developed from rediae and belonging to Petasiger *sp.,* Paryphostomum *sp., and other undetermined species were found.*

Key words: Planorbidae – schistosome vectors – ricefields – ecology

There is wide recognition of the important influence of dams and related irrigation systems on the spread of human schistosomiasis, not only in the Neotropical region, but also in nearly all African dams (Volta, Victoria, Aswan, Kainji, etc.) (Jordan, 1975; Kaul et al., 1980).

The obvious south expansion of schistosomiasis (Paraense & Corrêa, 1987) and the presence of potential schistosome-vector species in northeastern Argentina (NEA), make the ricefields related to the Río De La Plata basin capable of becoming endemic centers of infection where human contact with such potential vectors is facilitated.

At present, Corrientes province is the first producer of rice, with 43,000 ha cultivated. According to Jetter (1979), the factors that favor this are: climatic conditions, kind of soil (well provided with change base, medium content of organic material and deficient drainage) and numerous sources of available water (Paraná river, tributaries, pools and spring water).

Although extensive surveys of these molluscs (Bonetto et al., 1982; Paraense, 1975b; Rumi, 1986; Rumi & Tassara, 1985) and of their fauna of larval trematodes (Ostrowski et al., 1990) have been carried out in NEA natural environment, up to now few punctual observations of planorbids have been done in the ricefields of Corrientes province, and there are no bibliographic antecedents.

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Since the building of the projected dams on the Río De la Plata basin may facilitate the appearance of schistosomiasis foci in Argentina through its irrigation systems, the aim of this work was to collect basic information about planorbids and their associated trematodes in those systems as a contribution to the development of possible strategies for the control of *Schistosoma mansoni.*

The importance of a survey of trematodes parasitizing the potential vectors of schistosomiasis *(Biomphalaria tenagophila, B. straminea* and *B. peregrina)* lies in the possible competitive interaction and/or biological control, natural or induced, involving the autochthonous larval stages and *S. mansoni,* as demonstrated by the studies of Lie et al. (1965, 1968), Lim & Heyneman (1972), and the excellent revisions by Combes (1982) and Paraense (1987).

The goals of this study were as follows: 1) to survey and estimate the relative abundance of planorbids in the ricefields of Corrientes province, and determine the presence of potential vectors of *S. mansoni;* 2) to survey physical and chemical characteristics of that kind of environment in order to identify the factors capable of influencing either positively or negatively the population dynamics of those molluscs; and 3) to estimate the infection rates by trematodes of the planorbids surveyed.

MATERIALS AND METHODS

Sixteen ricefields related to the Paraná river basin (except one in Santo Tomé Department) were surveyed during the period from sowing to harvesting, between November 1987 and March 1988. The ricefields were divided into two zones: the A zone, above the confluence with the Paraguay river, between Ituzaingó and the city of Corrientes; and the B zone, below that confluence, between Corrientes and Goya (Fig. 1). Thirty-six samples were taken during an average collection time of 46 min and a range of 15 to 105 min, according to their abundance.

Snails were collected with a perforated dipper 12.5 cm in diameter, and their relative abundance was estimated by number of individuals per hr. They were classified according to Paraense (1957a, b).

The samples were taken from the cultivated parcels, irrigation canals and the possible natural reservoirs of planorbids (marshes, ditches and water mill tanks) in the ricefields.

Biomphalaria specimens were isolated in 5 ml vials with water for detection of cercariae. When present, the latter were observed *in vivo,* using vital dyes (neutral red and Nile blue sulfate), dense mounting media (which slows down the cercarial movements), conventional optical microscope, stereomicroscope and camera lucida drawings. They were classified according to La Rue (1957) and named according to the system of "open nomenclature" (Odening, 1971) which uses the collective group name (Furcocercariae, Echinocercariae, Xiphidiocercariae) as the generic name.

The physical and chemical characteristics of the water from the ricefields were temperature, pH (using colorimetric pH-meter) and conductivity (using battery conductimeter). Besides, samples of water were taken with a peristaltic pump to measure the concentration of dissolved oxygen (Winkler's method), calcium, total iron, nutrients (nutrites plus nitrates and soluble reactive phosphorus), hardness, carbonates and bicarbonates, according to Standard Methods (1980) and Golterman et al. (1978). The results are shown in the Table.

Soil granulometry was characterized according to Carver (1971).

The samples of water from an environment treated with biocides and/or fertilizers were not used in the analysis of correlation (according to Pearson's equation).

The differences between A and B zones, with regard to the density of planorbids in general and *Biomphalaria* in particular, were submitted to analysis of variance, *F*-test and *t*-test to differentiate between the means.

RESULTS

Collected species and relative abundance – In all, eight species were found, belonging to two genera – *Biomphalaria* and *Drepanotrema* – each with four species: *Biomphalaria tenagophila, B. straminea, B. peregrina* and *B. orbignyi; Drepanotrema anatinum, D. lucidum, D. kermatoides* and *D. depressissimum.*

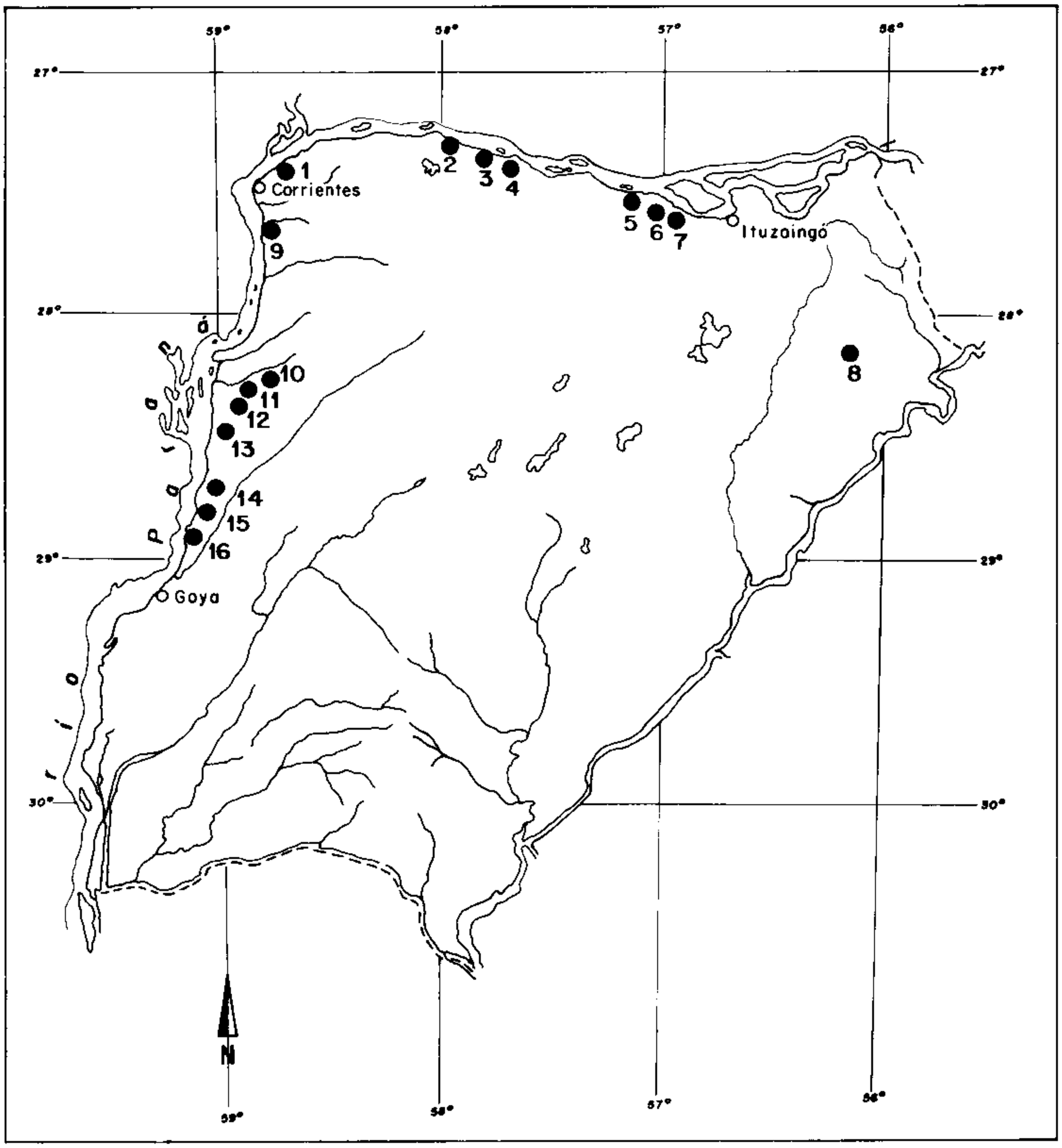

Fig. 1: surveyed ricefields along Ruta Nacional 12, from Goya to Ituzaingó (Corrientes province, Argentina). 1: Jachese, 2: Campanhar, 3: Machado, 4: Perrota Britos, 5: Ricefield km 1204, 6: Don Miguel, 7: Petirrojo and Santa Isabel, 8: INTA-EERA, 9: "El Sombrero", 10: "La Elisa", 11-12: Lagos, 13: Macchi, 14: Santinón, 15: Passe, 16: Chaín.

The genus *Biomphalaria* was more frequent and abundant. It was present in 52.8% of the samples with a mean of 200.39 individuals per hour of collection (ind/h). *Drepanotrema* was present in 36% of the samples with a mean of 38 ind/h.

The relative frequency of *Biomphalaria* species in the ricefields of the A and B zones is shown in Fig. 2: *B. straminea* was the most frequent in the A zone, as were *B. orbignyi* and *B. peregrina* in the B zone. In general, considering both zones, *B. orbignyi* was the most frequent (37.5%).

The average ind/h for each species of *Biomphalaria* in each ricefield of both zones is shown in Fig. 3, where the numerical predominance of *Biomphalaria* in the B zone can easily be observed. The most abundant species was *B. orbignyi*.

Comparing statistically the abundance of the genus *Biomphalaria* between A and B zones,

TABLE

Means and ranges of the environmental parameters registered in the ricefields of the A and B zones, Corrientes province, Argentina

	A Zone				B Zone			
	U		T		U		T	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
pH	6.5	6.2-6.8	–[a]	–	6.7	6.0-7.3	7.5	7.4-7.7
Conductivity μS/cm	66.6	60-70	67.5	60-75	187.1	40.600	397.5	315-480
Dissolved O_2 mg/l	4.3	1.80-6.85	2.5	1.55-3.44	5.7	3.84-6.76	3.63	3.60-3.67
HCO_3^- mg/l	27.2	26.5-28.1	27.9	26.3-29.6	86.2	28.8-178.0	–[b]	–
Ca^{++} mg/l	3.27	3.20-3.40	2.8	2.40-3.20	16.1	3.40-41.50	24.0	3.0-45.0
Hardness	11.40	8.7-12.8	10.1	7.40-12.8	50.7	16-131	144.0	121-167
Total Fe mg/l	0.64	0.17-1.50	0.12	0.10-0.15	0.16	0.08-0.50	0.40	0.15-0.65
N+N mg/l	0.08	0.04-0.11	0.20	0.12-0.28	0.44	0.01-2.55	0.01	0.01-0.015
$N\text{-}NH_4$ mg/l	0.28	0.12-0.58	0.46	0.22-0.70	0.49	0.10-0.81	0.05	0.045-0.05
Soluble reactive P mg/l	0.01	0.00-0.02	0.01	0.00-0.02	0.05	0.00-0.13	0.22	0.06-0.39
n =	4		2		7		2	

T = ricefields treated with chemical agents; U = untreated ricefields.
[a] A single measurement: 6.2.
[b] A single measurement: 186.0.

highly significant differences were observed ($t = 3.36; d.f. = 7; P < 0.01$). In the same way, the abundance of each species was compared and significant differences were obtained for *B. peregrina* ($t = 2.99$) and for *B. orbignyi* ($t = 2.21$); and no significant differences for *B. straminea* ($t = 1.12$) and *B. tenagophila* ($t = 1.00$), for $P > 0.05$.

Comparing the density of planorbids in general in both zones, the differences were significant ($F = 16.79; d.f. = 21; P < 0.01$).

With regard to the associated snails, they were represented by rather few species: *Pomacea canaliculata* (Ampullariidae), *Gundlachia concentrica* (Ancylidae), *Stenophysa* cf. *marmorata* (Physidae) and *Lymnaea columella* (Lymnaeidae). *P. canaliculata* was most frequent, especially in the A zone, associated with planorbids in 32.14% of the samples, and more frequently with *Drepanotrema* than with *Biomphalaria.*

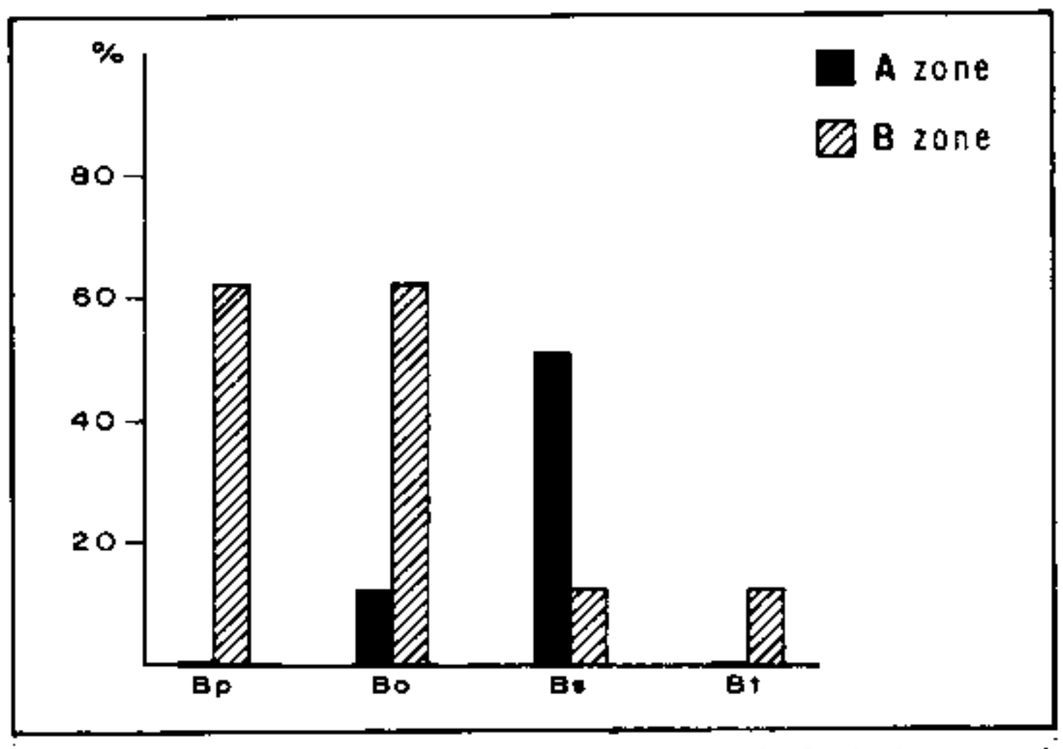

Fig. 2: percentage frequency of *Biomphalaria* in A and B zones. Bp = *B. peregrina*, Bo = *B. orbignyi*, Bs = *B. straminea*, Bt = *B. tenagophila.*

Environmental factors and their correlation with the Biomphalaria *populations* – Significant correlations were observed between the relative density of the genus *Biomphalaria* and the following environmental parameters, for the critical values of r, $d.f. = 8$, $P < 0.01$: conductivity ($r = 0.97$), hardness ($r = 0.90$), calcium

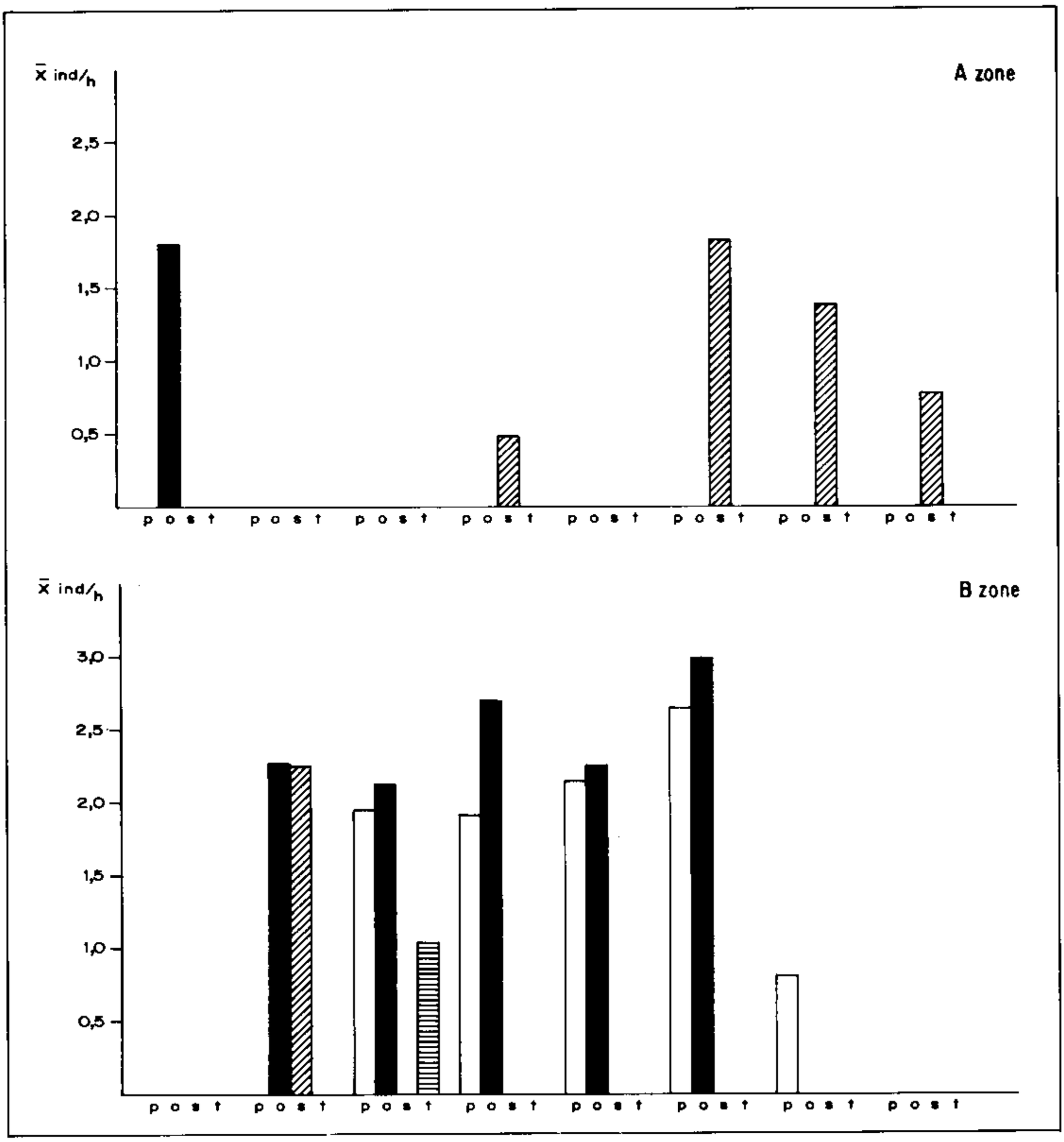

Fig. 3: mean number of individuals collected per hour (ind/h) by species and by ricefields in A and B zones. p = *Biomphalaria peregrina*, o = *B. orbignyi*, s = *B. straminea*, t = *B. tenagophila*.

($r = 0.86$), nitrites plus nitrates ($r = 0.87$), ammonium ($r = 0.71$), and bicarbonates ($r = 0.80$). Negative correlation was observed with water temperature ($r = -0.57$, $d.f. = 15$, $P < 0.05$). For correlations, see Fig. 4.

Correlations between the relative density of planorbids in general and the above-mentioned parameters showed similar significances for $P < 0.01$, including the temperature at this level of probability: conductivity ($r = 0.97$), hardness ($r = 0.90$), calcium ($r = 0.85$), nitrites + nitrates ($r = 0.86$), ammonium ($r = 0.71$), and temperature ($r = 0.60$).

When correlations between environmental parameters and each species of *Biomphalaria* were evaluated, only the most frequent species, *B. orbignyi* and *B. straminea*, were considered. In this respect, *B. orbignyi* showed more significant correlations, with the following r values: conductivity 0.82, temperature –0.59, calcium 0.70, bicarbonates 0.82, nitrites + nitrates 0.88, hardness 0.80, ammonium 0.88. *B. straminea* showed no significant correlation with the mentioned parameters.

No significant correlation was observed between the relative density of the genus

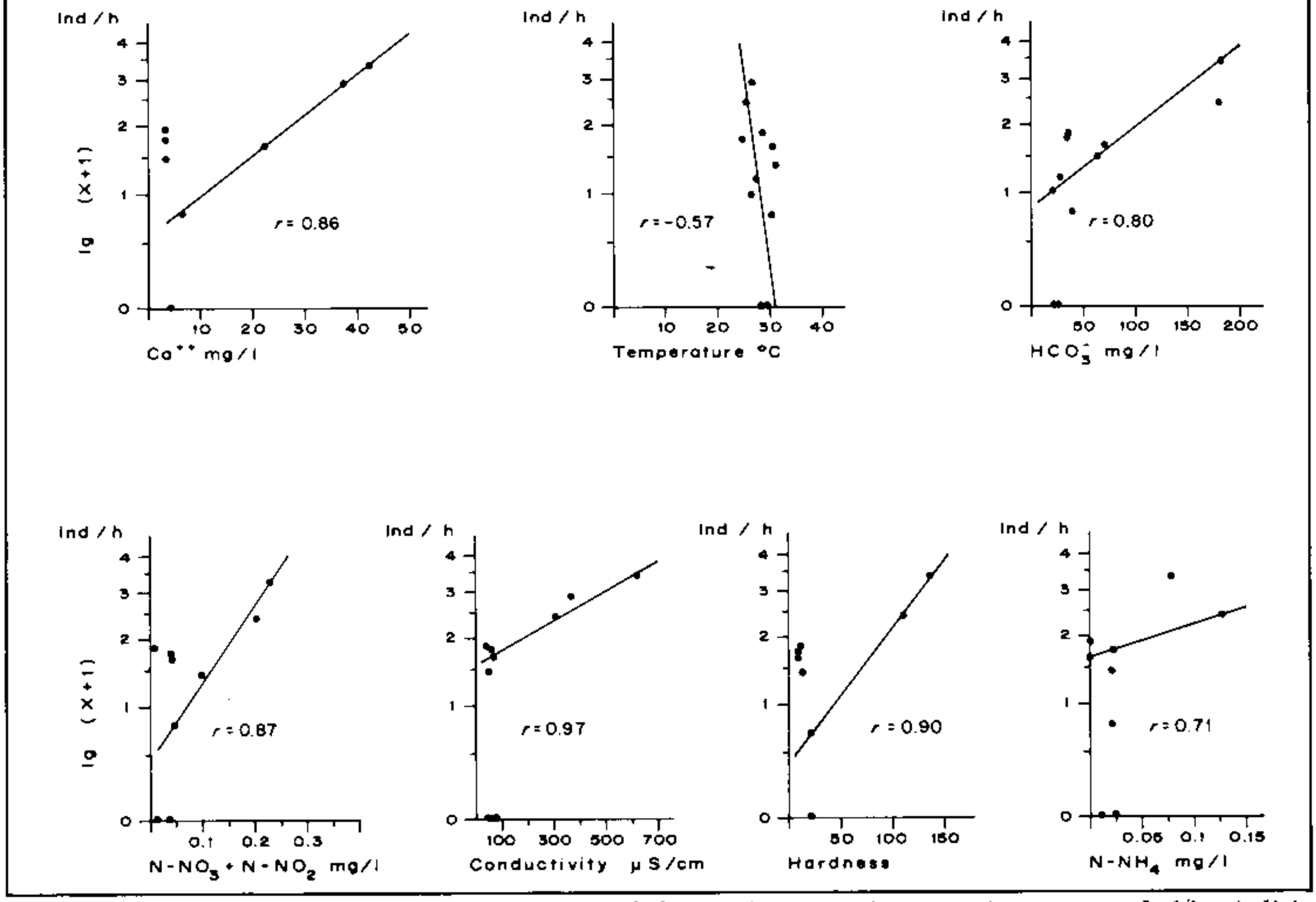

Fig. 4: correlations with significant *r* between *Biomphalaria* and some environmental parameters. Ind/h = individuals per hour.

Biomphalaria, each of its species, and planorbids in general, with total iron, phosphates and pH (which ranged from 6 to 7.7).

Granulometric analysis showed the soil of the ricefields to be mainly clayey, as observed by Melgar (1983).

When comparing the granulometry of water reservoirs, irrigation canals and the ricefields proper, a tendency was observed to a decrease, in that order, in the size of particles, as in the following examples:

	Sand %	Slime %	Clay %
Santo Tomé			
Storti dam	19	35	46
Irrigation canal	3	39	58
Ricefield	–	29	71
Santa Isabel (Ruta 12, km 1167)			
Irrigation canal	11	34	55
Ricefield	4	46	50

No correlation was observed between soil granulometry and abundance of planorbids.

Most irrigation canals, except those very recently filled, presented regional hydrophilic vegetation: *Ludwigia peploides, Sagittaria montevidensis, Hydrocotyle ranunculoides,* etc. This was not observed in the ricefields proper which, in general, had no hydrophytes. For this reason, it is not surprising that planorbids were more abundant in irrigation canals than in ricefields.

Trematode-vector associations – In A zone, 9.6% of 62 *B. straminea* examined shed cercariae: Furcocercariae of *Diplostomum* sp., 6.4%; Echinocercaria VII (*Petasiger* sp.), 1.6%; and Echinocercaria III (cf. *Paryphostomum* sp.), 1.6%. In B zone, 50% of 18 *B. orbignyi* shed cercariae: Notocotylidae, 5.6%, and Echinocercariae, 44.4%; and 20% of 10 *B. peregrina* shed Echinocercariae.

DISCUSSION

The above results show that three potential schistosome vectors – *B. straminea, B. tenagophila* and *B. peregrina* – are present in the biotopes investigated. Nevertheless, *B. orbignyi* appears as the most "successful" species in colonizing the ricefields of Corrientes province,

especially in the Corrientes-Goya section (B zone). This observation agrees with those by Bonetto et al. (1982), who found *B. orbignyi* to be the most frequent in the Middle Paraná. From the epidemiological point of view this is a fortunate occurrence, for according to Paraense (1975b) *B. orbignyi* is refractory at least to some *S. mansoni* strains.

As concerns the potential vectors, *B. tenagophila* appeared very sporadically, *B. peregrina* showed more considerable densities, and *B. straminea*, though the most frequent in the Corrientes-Ituzaingó section (A zone), showed a very low relative density. This may be due to several reasons: 1) In the 1987-88 period watering of the ricefields was begun too late, so that when the samples were taken – from late January to mid-February – the aquatic environments had only recently been established, giving the snails no time for regular breeding; 2) Water management – alteration of watering and drying – certainly impaired the early reproductive effort of the species, retarding colonization of the habitats, chiefly irrigation canals; as an example: in the Perrota Britos ricefield, where the second irrigation was completed in the first half of January, there was a high proportion of empty shells (95%) of small size (2 to 6 mm at most), indicating an early mortality (it should be mentioned that no biocides had been applied); 3) High temperatures were recorded in January 1988 – an accumulated maximum of 1140.5 °C, and over 30 °C in 25 days – by INTA (Instituto Nacional de Trabajos Agropecuarios), which negatively influences the reproductive potential and the survival of *Biomphalaria* species (Sturrock & Sturrock, 1972; Appleton, 1977). Those data support the negative correlation observed between *Biomphalaria* and temperature though, as concerns *B. straminea*, owing to its low density this relation was not too clear.

It would be recommendable to follow up *B. straminea* in the ricefields of Corrientes province to verify the regulatory capacity of the above-mentioned factors. That is, whether they are strong enough to keep the snail populations at a low density level throughout the rice cycle or, on the contrary, the breeding capacity of the population remainder succeeds in increasing significantly the populational density so as to possibly function as an efficient schistosome vector.

Our results, pointing to a significant correlation between *Biomphalaria* densities and conductivity, hardness, and calcium and bicarbonates concentration, which as a whole give an idea of the quantity of salts dissolved in the water inhabited by the snails, agree with the findings of Legendre et al. (1984), who also made a good synthesis of similar observations by other workers on several freshwater gastropods. As to the pH values, they varied from 6.0 to 7.7, which is within the usual range of 5.8 to 9.0 for planorbid snails (Pan American Health Organization, 1968).

The finding of Echinocercariae parasitizing the potential schistosome hosts (*B. tenagophila*, *B. straminea* and *B. peregrina*) opens the way to studies on competitive interactions between the Echinocercariae-producing rediae and the schistosome sporocysts, which may led to biological control of schistosomiasis (Lie et al., 1965). Important among those larval trematodes is the genus *Paryphostomum*, found in *B. straminea*, the rediae of which may act as facultative predators of *S. mansoni* sporocysts, as shown experimentally by Lie et al. (1968) using *P. segregatum* Dietz, 1909, parasite of *B. glabrata* and *B. straminea*. The rediae of this species predate not only sporocysts but also rediae of other echinostomatids which are predators in other combinations.

It would be of interest to proceed with the survey of the larval trematodes in the area concerned, since their knowledge could led to the assessment of their role as competitors of the intra-molluscan stages of *S. mansoni*.

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