Population Dynamics of Intermediate Snail Hosts of *Fasciola hepatica* and Some Environmental Factors in San Juan y Martinez Municipality, Cuba

Roberto Cañete, Mary Yong*, Jorge Sánchez*, Lin Wong*, Alfredo Gutiérrez*/+

Centro Provincial de Higiene y Epidemiología, Martí No. 159, Pinar del Río, Cuba *Laboratorio de Malacología, Instituto Pedro Kourí, Apartado 601, Marianao 13, Ciudad Habana, Cuba

The variation of abundances of intermediate snail hosts of Fasciola hepatica *in Cuba* (Fossaria cubensis *and* Pseudosuccinea columella) *was studied during one year under natural conditions at five sampling sites in San Juan y Martinez municipality, Pinar del Rio province, Cuba. The effect of some environmental variables on the lymnaeid abundances was also studied. A canonical correspondence analysis showed that both species do not generally occur together in the same habitat and that most factors affect them in an opposite fashion, although both of them correlate positively through time to the diversity of the habitats.* F. cubensis *prefers the sites that are in or closer to the city whereas* P. columella *is more abundant in rural sites. Lymnaeid abundances are mainly affected by nitrite and nitrate concentrations as well as by the abundance of the thiarid* Tarebia granifera. F. cubensis *is more abundant in polluted habitats with low densities (or absence) of* T. granifera *whereas* P. columella *prefers cleaner habitats and can coexist with the thiarid, even at its higher densities. The implications of divergent preferences of the two lymnaeids for the control of fasciolosis are discussed.*

Key words: *Fasciola hepatica* - *Fossaria cubensis* - *Pseudosuccinea columella* - Lymnaeidae - canonical correspondence analysis - Cuba

In Cuba fasciolosis is regarded as one of the most important parasitic diseases with both economical, showing high prevalence in cattle (Mauri 1972, Verdecia et al. 2001) and medical significance (Kourí 1948**,** Brito et al. 1987, Millan et al. 2000). This disease is caused by the liver fluke *Fasciola hepatica*, which needs a lymnaeid snail as intermediate host to complete its life cycle. Only two lymnaeids are known to be present in Cuba: *Fossaria cubensis* and *Pseudosuccinea columella*. Both species can host larvae of the trematode, but only the former has been found naturally infected in the island. Currently, only the curative aspect of the disease is taken into account by a chemotherapy treatment of the final host (Ritchter et al. 1999, Millan et al. 2000). No integrated program aiming at stopping or at limiting the transmission of the parasite, associating the control of intermediate snail hosts to other control strategies is currently carried out at the national level.

The development of an effective strategy of integrated control, with the precondition of a good knowledge on the epidemiology of this parasitosis requires including, among other things, the study of population dynamics of intermediate hosts and its relation to environmental factors.

+Corresponding author: Fax: 53-7-2046051. E-mail: alfredo@ipk.sld.cu
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This study had as objective to determine the variations of the abundance of *F. cubensis* and of *P. columella* as well as their relationship with various factors of the environment such as certain physico-chemical parameters of the water, the snail diversity and the abundance of other snail species.

MATERIALS AND METHODS

The field work was carried out in the municipality San Juan y Martinez, located south of Pinar del Rio province. Recently, high cattle infections by *F. hepatica* were referred in this locality as well as two human cases (which appear in the files of the local center of hygiene and epidemiology). Five sampling sites were selected in various water collections: (A) a culvert of residual waters from houses located close to the dispensary of the city; (B) a site on the river which crosses the city; (C) a drainage canal from a vegetable plantation located in the city; (D) a site on a small river outside the city and close to the small village of El Cañón; and (E) a small pond that originated and is fed by the water of an irrigation system. The sites A, C, and D are in or close to the city, whereas the sites B and E are more rural and with no or a little contact with the local population. Sampling took place once a month during one year (from April 2000 to May 2001)

The species abundance was estimated by collecting snails using a sieve and forceps, always by the same collector during 15 min; the abundance was then measured as the number of snails collected for each 15-min sampling. The snails were carried to the laboratory in plastic boxes and then identified and counted. The following environmental variables were measured: water temperature, pH, total hardness, nitrites, and nitrates. The snail diversity at each sampling was estimated by the index of

Shannon and Weaver (H') and its relationship to lymnaeid abundances was determined by a nonparametric correlation analysis (Spearman correlation index).

The relation between environmental variables and species abundances was determined by a canonical correspondence analysis (Ter Braak 1986).

RESULTS

In addition to the two lymnaeids, other snail species were found in the five studied sites: *Pomacea poeyana*, *Physa cubensis*, *Pyrgophorus coronatus*, *Corbicula fluminea*, and *Tarebia granifera*.

The abundances of the two lymnaeids, as well as the diversity index of Shannon-Weaver (H') show irregular fluctuations in all sites (Fig. 1). No season, rain or dry, seems to be more or less favorable for any of these species during the study period.

The results of the canonical correspondence analysis are presented in Table I and Fig. 2. The first three axes explain a 40% of the variance and 90% of the constrained variance. The species–environment correlations are higher than 0.5.

The two lymnaeids occupy opposite quadrants in the three ordination diagrams. The effect of variables like the temperature and total hardness is variable concerning the axis arrangement: in the ordination of axes 1 and 2 as well as of axes 2 and 3 the temperature and total hardness exert a positive influence on *P. columella* and negative on *F. cubensis*; in the ordination of axes 1 and 3 the effect is the opposite. The effect of pH, nitrites, and nitrates is always negative on *P. columella* and positive on *F. cubensis*, except in the ordination of axes 1 and 3, where the effect

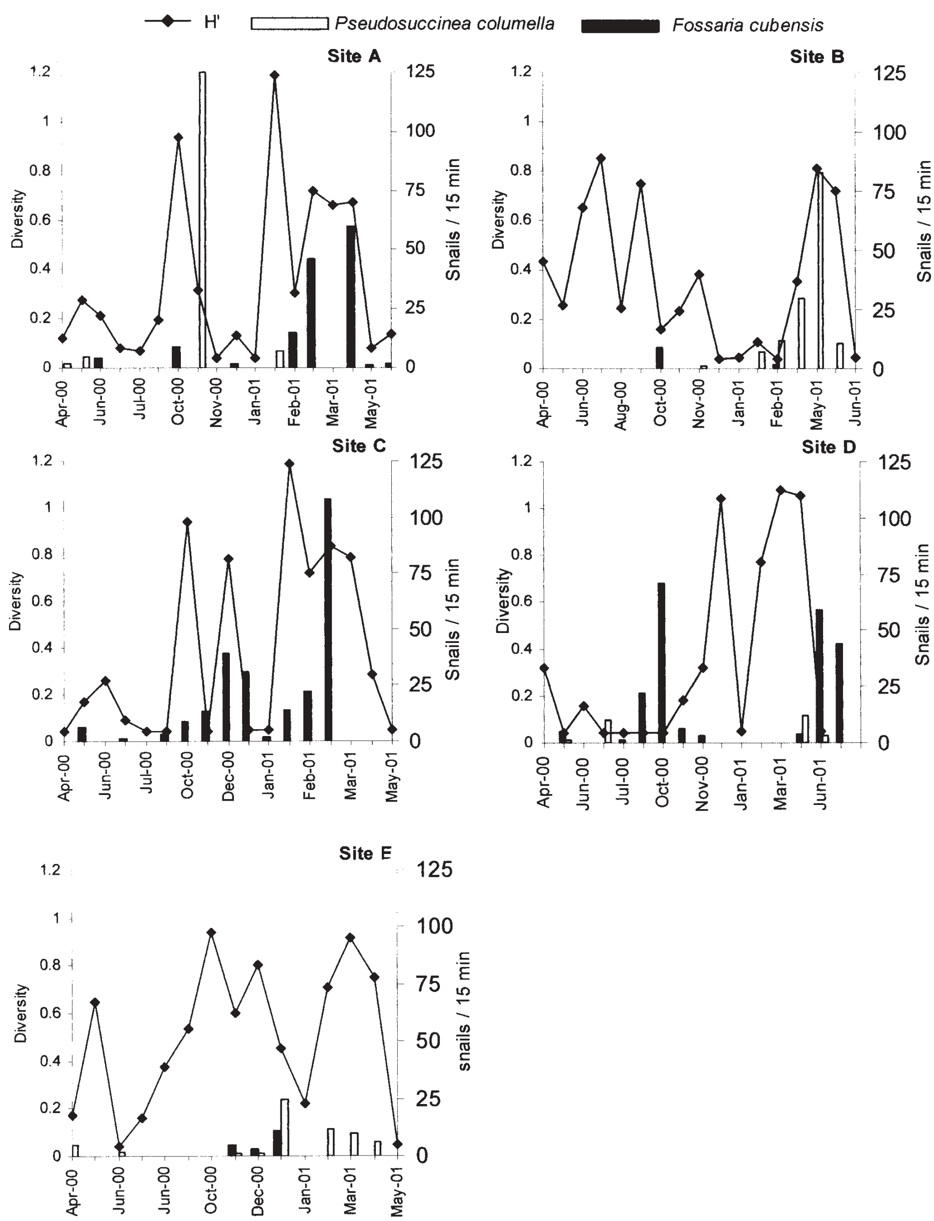

Fig. 1: abundances (snails/15 min) of lymnaeids and Shannon diversity index (H') in five sampling sites of San Juan y Martinez municipality, Pinar del Río, Cuba, during a one-year study.

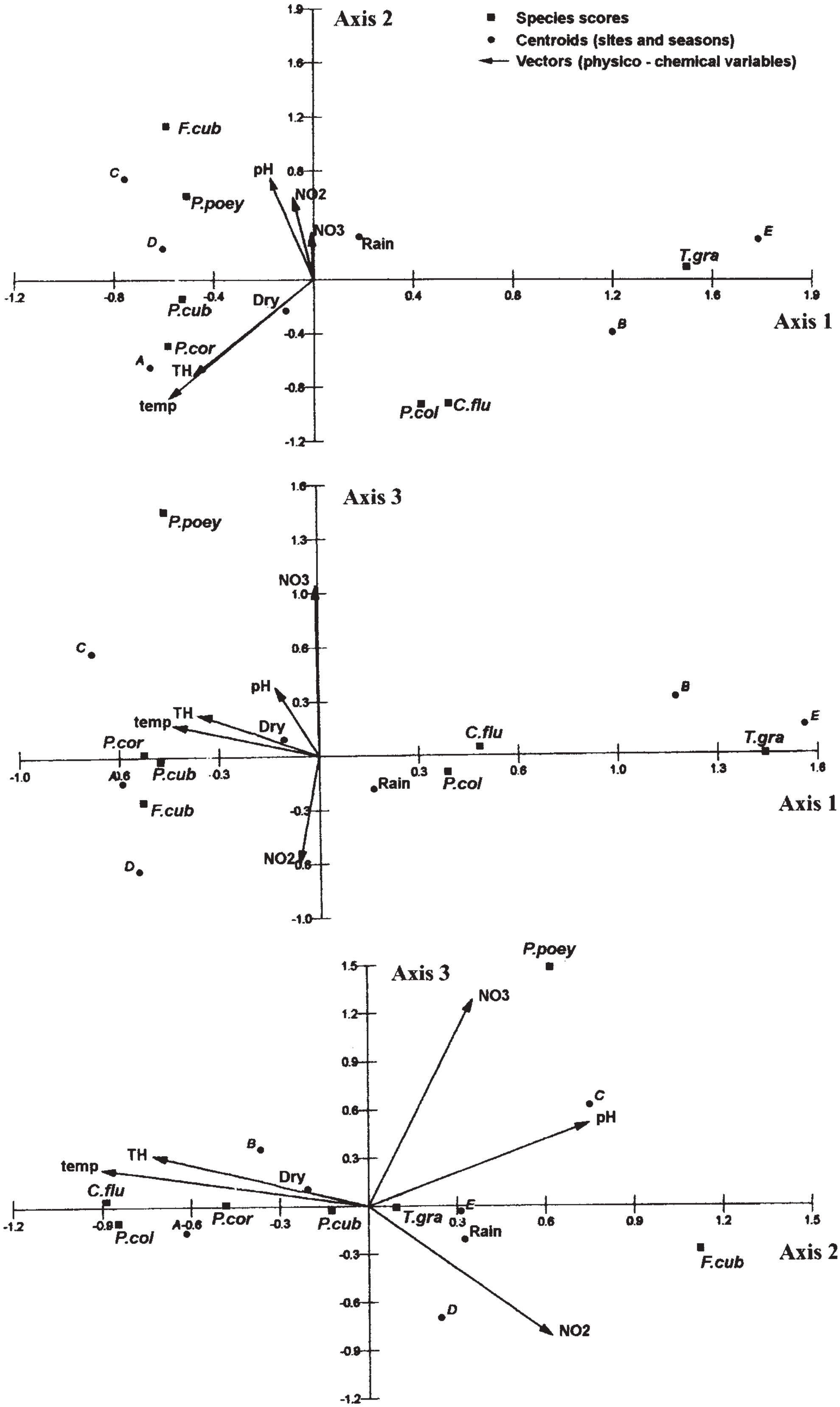

Fig. 2: ordination diagrams of the canonical correspondence analysis performed on five sites of San Juan y Martinez municipality, Pinar del Rio, Cuba. Physicochemical variables of the water are represented by their vectors and the sites (A, B, C, D, E) and seasons (Rain and Dry) are represented by their centroids. Species: *Fossaria cubensis* (F.cub); *Pomacea poeyana* (P.poey); *Tarebia granifera* (T.gra); *Physa cubensis* (*P*.cub); *Pygophorus coronatus* (P.cor); *Corbicula fluminea* (C.flu); *Pseudosuccinea columella* (P.col). Physico-chemical variables: pH (pH); nitrites (NO2); nitrates (NO3); total hardness (TH); temperature (temp)

TABLE I

Eigenvalues and percentages of the variance, obtained with the canonical correspondence analysis performed at five sites of San Juan y Martinez municipality, Pinar Del Rio, Cuba

	Axis 1	Axis 2	Axis 3
Eigenvalues	0.489	0.103	0.053
Percentage	30453	6.441	3.317
Cumulative percentage	30453	36.894	40211
Cumulative constrained percentage	69.309	83.968	91.517
Species–environment correlations	0.857	0.662	0.582

of nitrates is null on *P. columella* and negative on *F. cubensis*. It is also worth noting the association of *F. cubensis* with the urban sites A, C, and D, while *P. columella* is associated with the rural sites B and E, to which the prosobranch *T. granifera* seems also strongly associated.

The Spearman correlation coefficient between the index of diversity of Shannon-Weaver (H') and the lymnaeids abundances show a positive and significant association for the two species (Table II), yet this relationship is stronger for *F. cubensis* in the urban sites and for *P. columella* in the rural sites (data not shown). Curiously, the correlation between the abundances of lymnaeids and those of *T. granifera* show a positive and significant association for *P. columella* but negative and significant for *F. cubensis*. This negative effect of the thiarid on *F. cubensis* is still present when the same analysis is repeated considering only the urban sites (data not shown).

DISCUSSION

Of all the environmental factors which associations with lymnaeids were evaluated in this study, the abundances of *T. granifera* and of lymnaeids themselves show a very strong relationship. The nitrite concentrations and the anthropization of the sampling site are also strongly associated with the abundance of lymnaeids. The effect of pH is also constant for all diagrams but values alternate between 7, 7.5, and 8 and only one sampling had a value of 8.5 (data not shown). Therefore, this variable does not seem to affect lymnaeid abundances in an important way in this study since its range is well within the snail tolerance (Perera 1996). Other factors like the temperature and total hardness seem to play some important role but their association with the variations of lymnaeids' abundance changes from one diagram to another. That is perhaps the consequence of the influence of another more important factor, not measured in this study, such as for example the abundance of aquatic plants (Perera 1996). The variation range of these factors could also be one of the reasons why their association with the lymnaeid abundance was either weak or unstable. It is possible indeed that their concentrations do not reach the thresholds constituting the limiting values for lymnaeids. According to Moens (1981) a closely related species, *F. truncatula*, can tolerate a very broad spectrum of water physicochemical parameters, which enables it to occur in microhabitats where the fluctuations of these factors are very marked. If the Cuban lymnaeids have similar preferences, the density dependent factors (i.e life-history traits, the impact of the other species, food, etc.), should exert a more important influence on the variations of abundance than the density independent factors (like the physicochemical parameters of water, climate, etc.). According to theoretical studies, chance plays an important role in the variations of a species' abundance in the edges of its distribution area. In the center of its area, when environmental conditions (climate, etc.) are optimal, the regulation of the population by density dependent factors plays a dominant role (Richards 1961).

Mutual exclusion between *F. cubensis-P. columella* had already been observed by Perera (1996) in some localities of the Isle of Youth, of Santa Clara, and in Lake Hanabanilla. In all the analyses carried out by this author it can be systematically noted a negative correlation between the abundances of the two species, indicating that they rarely occur together in the same habitat. This "dislike for coexistence" was also evident in the present study. Our observations suggest that lymnaeids prefer to coexist with other snail species rather than with the other lymnaeid. An exception is perhaps the association between *F. cubensis* and *T. granifera*, where the correlations are always significant and negative.

The negative association of *T. granifera* with *F. cubensis* suggests relations of competition there too. This type of relationship was already shown between several species of Thiaridae and various species of pulmonates and these characteristics were exploited in various biological campaigns against the intermediate snail hosts of schistosomes (Perera et al. 1989, 1994, Pointier et al. 1989, Pointier 1983, 1991, 1993, Pointier & Guyard 1992). In Cuba, *T. granifera* is also a competitor of other prosobranchs like *Pomacea paludosa* (Gutiérrez et al. 1997). During our

TABLE II

Correlations between the abundances of lymnaeids and those of *Tarebia granifera* and diversity index of Shannon-Weaver, in the locality of San Juan y Martinez

Pair of variables	N	R	*p*
Pseudosuccinea columella - *Tarebia granifera*	82	0.326	0.002
Pseudosuccinea columella - H'	82	0.462	0.000
Fossaria cubensis - *Tarebia granifera*	82	-0.400	0.000
Fossaria cubensis - H'	82	0.476	0.000

N: number of observations; R: Spearman correlation coefficient; p: significance of the test; H': Shannon-Weaver diversity index study it was observed that *T. granifera* can displace the majority of other snails, becoming quickly the dominant species when conditions are favorable. Favorable conditions for this thiarid are met in permanent and stable habitats like the sampling sites B and E of this study. On the other hand, the positive association observed between *T. granifera* and *P. columella* is surprising and we do not have an explanation to this phenomenon. As a matter of fact, *P. columella* and *C. fluminea* are the only species not affected by *T. granifera* in our study.

The influence of nitrites is negative with *F. cubensis* but positive with *P. columella* and this was also the case in the analyses carried out by Perera (1996). Nitrite concentrations are good indicators of the degree of water pollution (Merk 1987) and Perera (1996) points out that *F. cubensis* shows high densities in sites where contamination of water by domestic or industrial effluents takes place. However, *P. columella* has been found in habitats with obvious signs of pollution as well, but always in absence (or in the presence of low densities) of *F. cubensis* (observations not published). These evidences suggest that the effect of interspecific competition is probably more important than the effect exerted by nitrite concentrations.

Another factor of importance associated with the lymnaeid abundance is the proximity of the sites to the city. Indeed, during our study *F. cubensis* was more frequently collected in more urban (and polluted) sites, whereas *P. columella* was more abundant in the rural sites, which evidently showed less signs of pollution. The presence of *F. cubensis* in the more anthropic sites may be related to a better tolerance of this species for the polluted habitats (Perera 1996).

The larger distribution of *F. cubensis* in Cuba seems to be related to an older presence of this species in the island. *P. columella*, however, is presumably of a more recent introduction and have not had the possibility yet of colonizing all the habitats available (Yong 1998). The colonization by *F. cubensis* of urban habitats confers this species a higher probability of contact with domestic animals, which constitute the main source of *F. hepatica* eggs. This is possibly the cause of not having yet found any *P. columella* infected in nature with the parasite in Cuba.

The different ecological preferences observed for the two lymnaeids indicates that strategies for the control of the liver fluke in Cuba should always consider the snail host species present as well as the characteristics of the habitat. Even though no evidence of natural infection has so far been found in *P. columella*, high infection rates for this species have been observed by us in laboratory exposures. Contrary to *F. cubensis*, where egg-laying cease before the onset of the cercaria-shedding period in infected snails (Gutiérrez et al. 2001), *P. columella* can lay eggs even while shedding cercaria (Gutiérrez et al. 2002). In addition, *P. columella* has been found naturally infected in Brazil and Australia (Ueta 1980, Boray et al. 1985, Oliveira et al. 2002) and its invasive nature has been observed in places like the island of Guadeloupe (Jean P Pointier, pers. commun.). Incorrect control strategies could lead to the colonization of *F. hepatica* transmission sites by *P. columella*, resulting in an increase rather than a decrease of the prevalence of the parasite in Cuba.

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