Life-history Traits of *Fossaria cubensis* (Gastropoda: Lymnaeidae) under Experimental Exposure to *Fasciola hepatica* (Trematoda: Digenea)

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The effect of exposing the lymnaeid snail Fossaria cubensis *to the trematode* Fasciola hepatica *on the snail population's life-history traits was studied under laboratory conditions. Exposed individuals showed a lower survival rate than control snails, although from week 7 onward a slower decrease of this parameter in relation to the control group was observed. There were higher values of fecundity rate for the controls compared to the exposed group except during weeks 9, 10, 11 and 12, which was the time that followed the period when almost all of the infected snails died. Both the intrinsic and finite rates of natural increase were significantly higher for the control group, but exposed snails still attained a lower mean generation time. Age-specific trade-offs were found, mainly for the weekly increase in size* versus *the number of eggs per mass, the weekly increase in size* versus *the number of viable eggs per mass, the number of masses* versus *the hatching probability and the number of eggs* versus *the hatching probability. All these negative associations were significant for juveniles of both control and exposed snails and not for adults; however, exposed young individuals exhibited much higher values of the correlation coefficient than control animals.*

Key words: *Fasciola hepatica* - *Fossaria cubensis* - interaction host/parasite - life-history traits

The hermaphrodite lymnaeid snail *Fossaria cubensis,* is the principal intermediate host of the trematode *Fasciola hepatica*, the causal agent of fasciolosis in animals and humans in Cuba.

It is widely known that trematode parasitic infection cause damage in intermediate snail hosts. Some authors have reported that parasites decrease the survivorship and fecundity of hosts, even castrating individuals (Anderson & Crombie 1984, Minchella 1985, Woolhouse & Chandiwana 1989) and, at the same time, induces a rapid body growth (Jong-Brink 1990). Others have demonstrated the effect of the infection on the foraging behavior of the snail, which increases the likelihood of the transmission to the final host (Levri & Liveli 1996).

The reproductive consequences of parasitism have shown a physiological basis, with parasites acting as regulators of the endocrine system of the snail. Some observations point out that the infection causes a distinct increase of synthetic activity of the dorsal bodies, and that the dorsal body hormone (DBH), which is also released into the haemolymph, does not evoke the normal effect (Sluiters et al. 1984). Besides, several studies have demonstrated the existence of an agent in infected snails that inhibits both the effect of cafluxine, the hormone that stimulates the influx of Ca^{2+} into the mitochondria of the albumen gland cells and the synthetic activity of DBH in this gland (Jong-Brink et al. 1986, Jong-Brink & Elsaadany 1987, Jong-Brink 1990).

The existence of non-susceptible strains in natural snail populations is known as well. This intrinsic resistance has demonstrated a genetic basis (Richards 1973), and certain morphological (Cousin et al. 1995) and molecular (Miller et al. 1996) changes have been observed in the albumen gland of non-susceptible snails. The host-parasite compatibility varies with the geographic area from where both the parasite and the host originate (Lively & Jokela 1996, Haroun 1996).

In the present paper we compare a group of snails exposed to miracidia of *F. hepatica* with non-exposed snails. The objective of this study was to describe the effect of the snail exposure to the trematode, since the original population was involved in the transmission of a human fasciolosis outbreak in 1995. We also assessed the trade-offs that took place during the life cycle of both exposed and control animals.

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MATERIALS AND METHODS

The snails used in the present study belong to a population from La Palma municipality, Pinar del Río province, where an outbreak of fasciolosis occurred in 1995. The sampling area consisted of a temporary pond that presumably functioned as the transmission site.

The animals were collected using a hand sieve, covering the bottom as well as the vegetation present on the shores, and transported to the laboratory on wet filter paper inside plastic cases. Upon arrival at the laboratory all animals were placed in Petri dishes containing mud and green algae as food source.

A group of egg masses laid by the snails were deposited in declorinated tap water until hatching took place. A total of 60 newly hatched snails were used for the life tables experiment. Two days after hatching, 30 snails were exposed to infection with three miracidia of *F. hepatica* per individual, attaining a prevalence of 66.6%. Another group of 30 was used as control.

Both exposed and control animals were divided into pairs. Each pair was kept in a Petri dish and was raised until the last snail died. The culture was maintained following the technique developed by Sánchez et al. (1995). Counts of live and dead animals, number of egg masses, number of eggs and number of viable eggs as well as measures of the shell size were made upon a weekly basis. We defined week zero as the week during which the 60 snails in study were born.

After the experiment was completed, the following life-tables parameters were determined using the Software TABVID 2.0, especially designed by the laboratory of malacology, IPK (©1996): survival rate (lx), fecundity rate (mx), hatching probability (hx), net reproduction rate (Ro), mean generation time (T) and the intrinsic (r) and finite (λ) rates of natural increase (Margalef 1986). We also determined the growth intensity (G), defined as the maximum length attained divided by the life span in weeks. Non-age-specific life tables (Ro, T, r, λ, G, maximum shell size and life span) were compared between exposed and control animals using the Student-t test, since all parameters were normally distributed (Dmax < 0.20, $p > 0.15$, N = 30, Kolmogorov-Smirnov test). Spearman rank correlations (Siegel & Castellan 1988) were performed among traits to find specific trade-offs in younger (less than 10 weeks old) and older (more than 10 weeks old) snails.

RESULTS

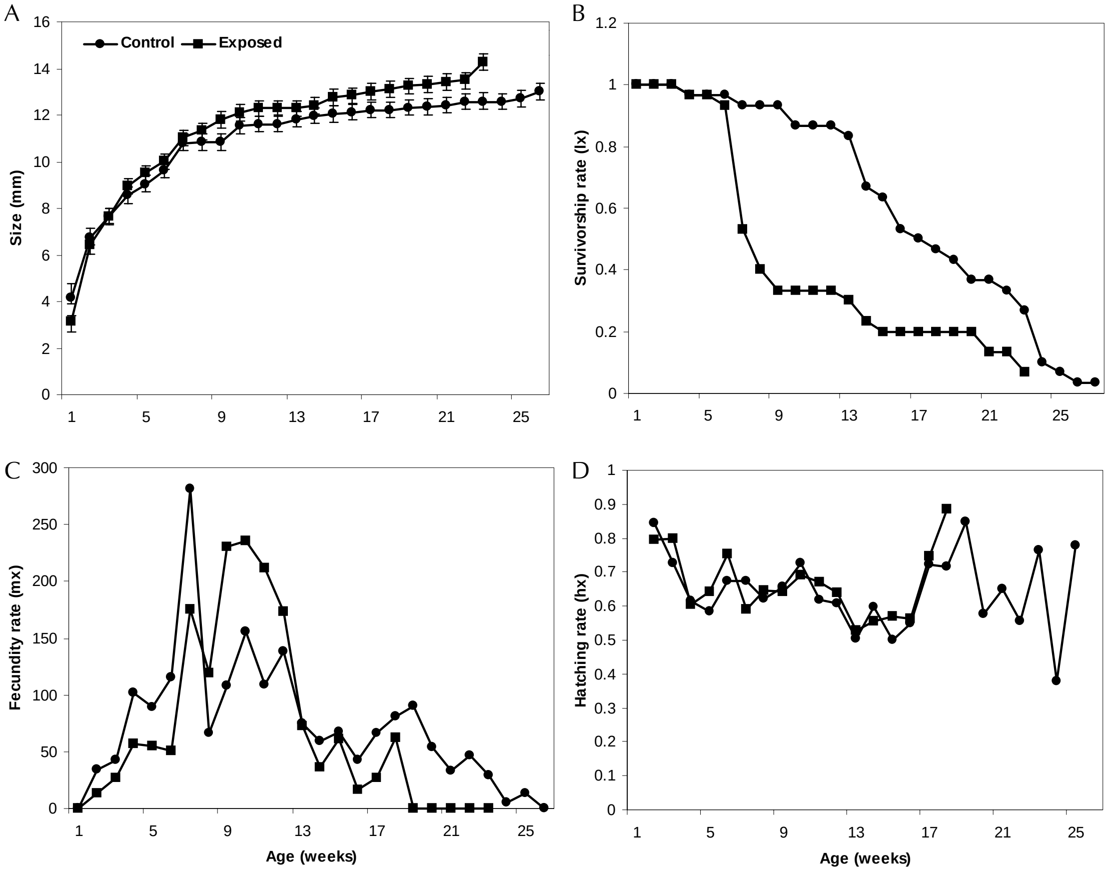
The individuals belonging to the control group lasted longer (27 weeks for the oldest snail) than the ones belonging to the exposed group (23 weeks for the oldest snail). The age-specific life tables are presented in the Figure. In general, exposed snails attained a greater shell size (mean value: 14.3 mm) than those of the same age in the control group (mean value: 13 mm). This difference was noted after week 7 and became more evident in older snails. At the same time, there was a rapid decline of the survival rate in the exposed group in week 7. After that age, only snails not susceptible to a dose of three miracidia were present in the exposed group.

Values of mx were higher for control snails than for exposed ones except for weeks 8, 9, 10, 11 and 12, which corresponded to the period that follows after the death of infected animals. The highest reproductive peak occurred in week 7 (281.1 viable eggs per snail) for the control group, and in week 10 for the exposed group (235 viable eggs per snail). Control individuals stopped laying eggs in week 25, whereas those of the exposed group ceased in week 19. There was a fast decline of the fecundity rate after the reproductive peak in both groups. The hatching probability did not differ significantly between groups for most of the life range, except for weeks 6 and 18, though differences were not as great as for the fecundity rate.

Almost all of the non-age-specific life tables were significantly different between groups (Table I). The maximum size attained by an average individual did not exhibit significant differences between groups; however, the snails of the exposed group manifested a significantly greater G than the control snails ($p < 0.001$). All of the reproductive parameters showed greater values in non-exposed animals than in the exposed group.

Spearman Ranks Correlation Coefficients (Table II) showed a negative and significant association of Ro and T with G for the group of control snails. This was the case for exposed snails as well, but there was also a strong negative correlation of the two rates of natural increase (intrinsic and finite) with the G in this group. All of the four reproductive parameters (Ro, T, λ and r) had a positive correlation with the maximum size and the life span, the strongest association being for the group of exposed snails.

Interactions of the weekly increase in size, number of egg masses per snail and number of egg per snail with the hatching probability, number of egg per mass and number of viable eggs per mass are presented in Table III for both young (less then 10 weeks old) and old (more than 10 weeks old) snails. The highest significant and negative correlations (Spearman Ranks Correlation Coefficient) were observed in the younger group of exposed snails. These significant interactions involved the weekly



Four age-specific life tables curves of the exposed and control groups of *Fossaria cubensis* from La Palma, Pinar del Río, Cuba. Vertical lines represent values of the standard deviation. A: growth; B: survivorship probability (lx); C: fecundity rate (mx); D: hatching probability (hx)

TABLE I

Values of seven life-history traits and Student's t-test for control and exposed individuals of *Fossaria cubensis*. Shell size is given in millimeters and life span in weeks

Group	Ro	T	λ	r	Max. shell size	Life span	Growth intensity
Control	1406.94	9.381	6.597	1.861	13.00	16.967	0.786
Exposed	688.7	7.240	4.480	1.440	14.3	9.80	1.340
t-Student	4.31^{a}	3.41^{a}	3.80^{b}	3.78^{b}	1.13$^{\text{n.s}}$	5.03^{b}	-5.45^{b}

Ro: net reproduction rate; T: mean generation time; λ: finite rate of natural increase; r: intrinsic rate of natural increase. *a*: $p < 0.01$; *b*: $p < 0.001$; n.s: $p>0.05$

increase in size *versus* the number of eggs per mass, the weekly increase in size *versus* the number of viable eggs per mass, the number of egg masses *versus* the hatching probability and the number of eggs *versus* the hatching probability. The younger control group exhibited the same four interactions, but they manifested to a lesser extent. The correlation between the weekly increase in size and the hx was significant only for the younger group of control snails. Interactions of the number of egg masses *versus* the number of eggs per mass, the number of egg masses *versus* the number of viable eggs and the number of eggs *versus* the number of viable eggs per mass were always positive and in some cases significant, the highest values being found for the adult group of exposed snails.

TABLE II

Values of the spearman ranks correlation coefficient of four non-age-specific life-history traits with the maximum size attained, the life span and the growth intensity for control and exposed snails of *Fossaria cubensis*. Numbers in bold represent significant trade-offs

Group	Trait	Maximum size	Life span	Growth intensity
Control	Ro	0.06	0.64^a	**-0.68**^{a}
	T	0.02	0.65^a	**-0.65**^{a}
	λ	0.42^a	0.19	-0.16
	r	0.40^a	0.15	-0.14
Exposed	Ro	0.88^a	0.85^a	**-0.85**^{a}
	T	0.87^a	0.89^a	**-0.91**^{a}
	λ	0.65^a	0.60^a	**-0.64**^{a}
	r	0.67^a	0.63^a	**-0.66**^{a}

Ro: net reproduction rate; T: mean generation time; λ: finite rate of natural increase; r: intrinsic rate of natural increase; *a*: $p < 0.01$

TABLE III

Values of the Spearman Ranks Correlation Coefficient between six life-history traits for control and exposed snails of *Fossaria cubensis*. Numbers in bold represent significant trade-offs

Group	Parameter	Hx	Eggs/mass	Viable eggs
Control (<= 10 weeks old)	W.I.S.	0.235^b	**-0.339**^{c}	**-0.293**^{c}
n = 135	Total masses	**-0.263**^{b}	0.207^a	0.16
	Total eggs	**-0.290**^{c}	0.748^c	0.659^c
Control (> 10 weeks old)	W.I.S.	-0.102	0.074	0.016
n = 127	Total masses	-0.141	0.249^b	0.126
	Total eggs	-0.163	0.730^c	0.506^c
Exposed (<= 10 weeks old)	W.I.S	0.082	**-0.574**^{c}	**-0.612**^{c}
n = 104	Total masses	**-0.374**^{c}	0.287^b	0.125
	Total eggs	**-0.409**^{c}	0.781^c	0.618^c
Exposed (> 10 weeks old)	W.I.S.	-0.082	-0.219	-0.215
n = 50	Total masses	0.226	0.356^a	0.395^b
	Total eggs	0.342^a	0.822^c	0.808^c

W.I.S: weekly increase in size; Hx: hatching probability; *a*: $P<0.05$; *b*: $p<0.01$; *c*: $p<0.001$

DISCUSSION

Both exposed and control snails had a longer life span than those observed in previous studies for this species in Cuba (Yong et al. 1995). This may be due to the procedure used to raise the snails in this particular experiment (two individuals per dish), avoiding the negative effects of high population density.

Differences in the age-specific life table curves agreed with the literature that states that, at least for the genus *Lymnaea* (Jong-Brink 1990) as well as for *Bulinus* (Jarne et al. 1996), parasitism decreases the survivorship and fecundity of the hosts. Jong-Brink (1990) and Ballabeni (1995) have also pointed out that parasitic infection stimulates the growth, inducing gigantism. We found the highest mortality rate on week 7 for the group of exposed snails (42 days after infection) possibly as a result of the cercarial emission of infected snails. However, the remaining snails had a slower decrease in survivorship rate after this age than the snails of the control group. The fecundity rate was also lower for the exposed animals before week 7 (when control snails had a reproductive peak), but the remaining snails showed higher values of this variable than control snails during weeks 9 to 12. As no snail, in the control group, laid eggs when its pair died, we do not consider the reproductive peak in the exposed group to be a result of the death of infected animals.

It is unlikely, from our data, that the infected snails increase the hatching probability of their eggs as a compensation for their lowered fecundity compared to non-exposed snails.

In spite of the Ro of the control group being twice that of the exposed one, the latter still attain high rates of natural increase (λ and r) compared to values reported in the literature for other limnaeids (deWitt & Sloan 1958). The reason for this is the low value in T that exposed snails exhibited, implying that infected animals have a shorter time to maturation to compensate for the lower fecundity. Nevertheless, the rates of natural increase attained by control snails were significantly higher than those exhibited by the exposed snails.

We also found that the maximum size attained did not differ between groups, possibly due to the size of exposed non-infected snails, but when this parameter was divided by the life span (obtaining G), significant differences were found. It is evident that a stimulation of growth does occur for the whole group of exposed snails. This enhanced growth may be a result of a host manipulation in order to increase the space available for parasite development. Ballabeni (1995) speculates that the induced gigantism may be a result of a host adaptation to the new adverse conditions. Gerard and Theron (1997) suggest two possible non-exclusive causes for the stimulation of growth in the *Biomphalaria glabrata-Schistosoma mansoni* system: an increase of the foraging effort of the infected snails and/or a reallocation of resources from a particular compartment of the host metabolism. It seems that both mechanisms are present in the *F. cubensis-F. hepatica* system and further studies shall be done in order to measure the foraging effort in infected snails.

Some interactions were observed between traits. The most interesting associations occur between the non-age-specific reproductive life traits (Ro, T, λ and r) and G. Even though there was a non-significant or positive association between the non-age-specific life tables and the maximum size attained, we found a negative significant correlation with G, the highest values being obtained for the group of exposed snails. It is obvious that the traits involved in this trade-off compete for the snail's energy and that in exposed snails this competition is much stronger than in the control group, the energy being directed in the former towards a fast increment in size.

When the age was taken into account to assess significant associations between life-tables, interesting trade-offs were obtained as well. Negative correlations were found mostly for younger snails (less than 10 weeks old), and these associations were also stronger in the group of exposed snails. The most significant interactions comprised those between the weekly increase in size and both the number of eggs per mass and the number of viable eggs per mass. The difference between control and exposed snails in the magnitude of these associations may be a result of the parasite competition with the host metabolic processes for the host's energy. There is also a trade-off between the number of eggs and H, which is also more intense in exposed snails, suggesting that as fewer eggs are laid, more energy be directed towards guaranteeing their viability. The younger group of control snails also manifested these interactions, although to a lesser extent. It seems that trade-offs are not important in older snails since the associations were either non-significant or positive in both the control and the exposed group.

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