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The presence of predators modifies the larval development of *Fasciola hepatica* in surviving *Lymnaea truncatula*

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Abstract

Experimental infections of *Lymnaea truncatula* with *Fasciola hepatica* were performed to study the consequences of the presence of predators (sciomyzid larvae or zonitid snails) on the characteristics of larval *F. hepatica* development in surviving snails. Controls consisted of infected snails that were not subjected to predators. Compared to controls, the survival rate at day 30 post-exposure, the duration of cercarial shedding, and the number of cercariae shed by surviving snails were significantly lower when predators were present in snail breeding boxes, whatever the type of predator used. In contrast, the prevalences of *Fasciola* infections in snails, and the length of time between exposure and the onset of cercarial shedding showed no significant variation. The progressive development of a stress reaction in surviving snails against predators during the first 30 days of experimental exposure to *F. hepatica* would influence snail survival during the cercarial shedding period and, consequently, the number of cercariae shed by the snails.

Introduction

Most major groups of animals include a few species that feed on *Lymnaea truncatula* (see Berg, 1964 or Taylor, 1965) and the list of predators varies with the behaviour of this amphibious snail. When *L. truncatula* is in the water, aquatic beetles, such as adult Dytiscidae or larvae of Sciomyzidae, Diptera, for example, kill any snail at or near the surface of the water (Knutson, 1976). Conversely, when snails emerge on land, other terrestrial predators, such as the snail *Zonitoides nitidus*, directly attack *L. truncatula* and consume large quantities of its tissues (Rondelaud, 1975). In both cases, the behaviour of surviving *L. truncatula* is modified so that snails either take refuge on land, or remain in the water (Rondelaud, 1978).

Predation on *L. truncatula* frequently results in snail mortality and can interrupt the larval development of *Fasciola hepatica*, as the lymnaeid species is known to be the main intermediate host of this digenean. However, the total destruction of a snail population by predators is unlikely and many snails are able to survive. Under such circumstances, two questions arise concerning the development of larval *F. hepatica*, namely: do predators influence the characteristics of *F. hepatica* larval development in surviving *L. truncatula*, and are these consequences similar when different types of predators (aquatic or terrestrial) are used? In an attempt to answer these questions, two experiments were performed by subjecting infected *L. truncatula* to the presence of predators (sciomyzid larvae or *Z. nitidus*) and by studying cercarial shedding.

Materials and methods

Two populations of *L. truncatula* inhabited road ditches at Masvaudier, commune of Saint Michel de Veisse, department of Creuse, and at La Poitevine, commune of Nieul, department of Haute Vienne (central France). These colonies were known to be devoid of any natural trematode infections as an examination of monthly samples of 50 adult snails over a period of two years revealed no larval forms. A total of 440 preadult snails,

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Table 1. The characteristics of seven groups of *Lymnaea truncatula* experimentally infected with *Fasciola hepatica* and subjected or not subjected to predators.

*Lymnaea truncatula**		Predators	
Locality	No. per group	Type	No. per group at onset of experiment
St Michel de Veisse	110	Controls	0
	110	First-instar larvae of *Tetanocera arrogans***	10
	110		20
	110	Preadult *Zonitoides nitidus***	10
Nieul	100	Controls	0
	100	Third-instar larvae of *T. arrogans***	6
	100	Adult *Z. nitidus***	10

*Two miracidia of *Fasciola hepatica* per snail for 4 h.
**Predators and snails were in contact for the first 30 days of the experiment.

4 ± 0.1 mm, high, were collected in June 1999 from Saint Michel de Veisse, and 300 other snails were collected in June 2000 from Nieul. Eggs of *F. hepatica* were collected from the slaughterhouse of Limoges from the gallbladders of heavily infected cattle and were incubated in complete darkness for 20 days at 20°C. First- and third-instar larvae of *Tetanocera arrogans* (Sciomyzidae) were collected from a road ditch at Courcelles, commune of Saint Michel de Veisse, department of Creuse, and *Z. nitidus*, 5 ± 0.2 mm wide, were collected from a swampy meadow, located at Landouge, near Limoges. The identification of adult *T. arrogans* (after their exit from puparia) was made using the systematic keys of Vala (1989).

Two experiments (the first in June 1999, and the second in June 2000) were performed using a total of seven snail groups. The population of *L. truncatula* used for each experiment, the number of snails per group, the type of predator used, and the number of predators per snail group are shown in table 1. The 740 snails used in these experiments were individually exposed to two *F. hepatica* miracidia for 4 h. After miracidial exposure, two groups (one per experiment) were used as controls and no predators were utilized. In the three other groups from the first experiment, five first-instar sciomyzid larvae, ten first-instar sciomyzid larvae, or five preadult *Z. nitidus*, respectively, were placed in each snail breeding box. In the last two groups from the second experiment, three third-instar sciomyzid larvae, or five adult *Z. nitidus*, respectively, were introduced. The choice of the number of predators used for each snail group and each breeding box was based on preliminary results obtained for the number of snails killed by each type of predator over a period of 30 days. The snails from the seven groups were subsequently raised for 30 days in polypropylene boxes 1 m by 55 cm and 15 cm high, with 50–55 snails per box (Augot *et al.*, 1996). Boxes were maintained under constant conditions of 20°C ambient temperature, and a diurnal photoperiod of 3000–4000 lux light intensity. At day 30 post-exposure (p.e.), each surviving snail was placed in a 35-mm diameter Petri dish, each containing 2–3 ml of spring water and a piece of lettuce. These dishes were maintained at 20°C. Each day, a cercarial count was performed and the water in the dish was changed until snail death.

The parameters studied included survival rates of snails at day 30 p.e., the prevalence of *Fasciola* infection, the length of time between exposure and the first cercarial shedding, the duration of shedding, and the total number of cercariae produced by each surviving snail. The number of predators surviving at the end of experiment and their transformation into subsequent-instar larvae or encased pupae (for sciomyzid larvae) were also considered. A comparison test of experimental frequencies and a one-way analysis of variance (Stat-Itcf, 1988) were used to establish levels of significance.

Results

Table 2 shows the results from the first experiment. Significant differences were noted for three parameters. Firstly, the survival rate of controls at day 30 p.e. was significantly higher ($P < 0.05$) than that recorded in each group subjected to predators. Another significant difference ($P < 0.05$) was also found between the rate noted in the group with *Z. nitidus* and those recorded in groups with sciomyzid larvae. Secondly, the duration of cercarial shedding was significantly greater ($F = 6.13$; $P < 0.01$) in controls than in the three other groups, whereas the mean duration in these last groups did not significantly differ. Thirdly, the number of cercariae was also higher ($F = 5.54$; $P < 0.01$) in controls than in the snails exposed to predators, whilst no significant variation between the mean values of these last groups was noted. No significant differences were found between the prevalence of *Fasciola* infections in the four snail groups, nor between the length of time between exposure and the first cercarial shedding. In the breeding boxes with Sciomyzidae, a total of 12 second- and one third-instar live larvae were noted, whereas two living *Z. nitidus* (one per box) were only found in boxes from the last snail group (data not shown).

Table 3 shows the results from the second experiment. Compared to controls, the survival rate of *L. truncatula* at day 30 p.e. was significantly lower ($P < 0.05$) in the two snail groups exposed to predators. Significant differences were also noted for the duration of the shedding period ($F = 7.15$; $P < 0.01$) and the number of cercariae per snail ($F = 6.24$; $P < 0.01$) which were also lower in the groups

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Table 2. Characteristics of *Fasciola hepatica* infections in *Lymnaea truncatula* subjected or not subjected to first-instar larvae of Sciomyzidae, or to preadult *Zonitoides nitidus* (first experiment).

		Number of predators per group		
		First instar larvae of *Tetanocera arrogans*		Preadult *Zonitoides nitidus*
Parameters	Controls	10	20	10
No. of survivors at day 30 p.e. and proportion surviving (%)*	78 (70.9)	37 (33.6)	31 (28.1)	23 (20.9)
No. of cercaria-shedding snails and prevalence of infection (%)	53 (67.9)	27 (72.9)	22 (70.9)	15 (65.2)
Length of time between exposure and the first cercarial shedding (days): mean ± SD	53.2 ± 7.1	61.3 ± 12.7	59.4 ± 10.3	58.2 ± 13.5
Duration of shedding period (days): mean ± SD	21.2 ± 6.4	11.2 ± 6.0	8.3 ± 6.3	7.5 ± 5.7
No. of cercariae shed per snail: mean ± SD	87.2 ± 41.3	35.3 ± 15.8	24.3 ± 21.8	19.7 ± 16.7

*110 snails per group at miracidial exposure.

exposed to predators. In contrast, there were no significant differences between values recorded in the sciomyzid- and zonitid-subjected groups for each of the aforementioned parameters. There were no significant differences between the prevalence of *Fasciola* infections in the three snail groups, nor between the length of time between exposure and the first cercarial shedding. In breeding boxes with the Sciomyzidae, one larva pupated in the water, whilst others were always third-instar larvae. Two living *Z. nitidus* (one per box) were found in the boxes with this predator (data not shown).

Discussion

Despite slight fluctuations in mean values, snail survival at day 30 p.e., the duration of shedding, and the number of cercariae released by surviving snails were clearly lower when predators were present in the snail breeding boxes. If survival rates are more easily explained by predation, the lower duration of shedding and the number of cercariae are more difficult to interpret. As these results were similar in the two experiments, it is difficult to explain these findings either by the type of predator (terrestrial, or aquatic) used, or the modifications recorded in the behaviour of surviving snails (Rondelaud, 1978). The results from the two experiments might be explained by the occurrence of a stress reaction that surviving snails would progressively develop during the first 30 days of the experiment when predators were present. An argument in support of this hypothesis was the recording by Abrous *et al.* (2001) of enhanced prevalences of *F. hepatica* infections in *L. truncatula* stressed by a cold-shock, for example, just before miracidial exposure. Stress reactions due to the presence of predators might ultimately affect snail survival and, in turn, the number of emerging cercariae.

On day 30 p.e. a decrease in the number of living sciomyzid larvae from the first experiment might be explained by competition occurring between these larvae for the same snails, as suggested by Berg (1964). However, such competition would be confined to first-instar larvae,

Table 3. Characteristics of *Fasciola hepatica* infections in *Lymnaea truncatula* subjected or not subjected to third-instar larvae of Sciomyzidae, or to adult *Zonitoides nitidus* (second experiment).

		Number of predators per group	
Parameters	Controls	6 third-instar larvae of *Tetanocera arrogans*	10 adult *Zonitoides nitidus*
No. of survivors at day 30 p.e. and proportion surviving (%)*	73 (73.0)	15 (15.0)	21 (21.0)
No. of cercaria-shedding snails and prevalence of infection (%)	52 (71.2)	10 (66.6)	15 (71.4)
Length of time between exposure and the first cercarial shedding (days): mean ± SD	54.3 ± 8.2	60.5 ± 9.7	54.3 ± 11.7
Duration of shedding period (days): mean ± SD	24.3 ± 7.1	8.6 ± 4.2	9.3 ± 8.3
No. of cercariae shed per snail: mean ± SD	93.5 ± 64.3	10.5 ± 8.7	14.2 ± 10.1

*100 snails per group at miracidial exposure.

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as all third-instar larvae were found to be alive in the second experiment. The reduced number of living *Z. nitidus* (one per box) in the four snail breeding boxes is likely to be explained by the feeding behaviour of this predator which actively consumed other snails of its own population (Didier & Rondelaud, 1989).

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