Multiple infection of *Hydrobia stagnorum* (Gmelin) with larval Trematodes

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Summary.

Analysis of double infections of *Hydrobia stagnorum* (Gmelin) with larval trematodes leads to the following conclusions:

1. — negative interaction occurs when both species have the same precercarial stage (rediae or sporocysts);
2. — negative interaction is generally absent when these precercarial stages are different (redia + sporocyst);
3. — the few exceptions on 2. could be a result of predation by the redia on the sporocyst;
4. — the negative interactions are most likely the result of competition for the same type of food.

Résumé.

Infection multiple d'*Hydrobia stagnorum* (Gmelin) avec des larves de Trématodes.

Une analyse des infections doubles par des trématodes larvaires chez *Hydrobia stagnorum* (Gmelin) nous amène aux conclusions suivantes :

1. — une interaction négative existe quand les deux espèces ont le même stade précercarien (rédies ou sporocystes) ;
2. — cette interaction négative est généralement absente quand ces stades sont différents (rédie + sporocyste) ;
3. — les quelques exceptions sur 2. pourraient être le résultat de prédation des sporocystes par les rédies ;
4. — les interactions négatives sont très probablement le résultat d'une compétition pour le même type de nourriture.

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Introduction

Several authors have observed multiple (mostly double) infection of the first intermediate host with larval trematodes (Dubois, 1929 ; Rees, 1932 ; Cort *et al.*, 1937 ; Lysaght, 1941 ; Wikgren, 1956 ; Cort *et al.*, 1960 ; Bourns, 1963 ; Kendall, 1964 ; Bowers, 1965 ; Lie *et al.*, 1966 ; Probert, 1966 ; Heyneman and Umathevy, 1968 ; James, 1969 ; Vernberg *et al.*, 1969 ; Werding, 1969 ; Anteson, 1970).

Generally speaking, the frequency of the double infections was lower than could be expected, assuming that the distribution of the infective stages would be random. In some rare cases, the opposite was true (Ewers, 1960 ; Bourns, 1963 ; Vernberg *et al.*, 1969).

It has also been noted repeatedly that echinostomatid species seldom occur in multiple infections (Donges, 1972).

In the present study, an analysis was made of the multiple infections occurring in a population of *Hydrobia stagnorum*.

Material and methods

From August 1969 to December 1970, 3,400 specimens of *H. stagnorum* were examined, and from January 1972 to August 1974 again 12,923. The snails were collected in a shallow brackish-water pond in the North of Belgium, called Dievegat. 13 trematode species used this snail as first intermediate host. For a description of the habitat and a survey of the life-cycles we refer to a previous paper (Vaes, 1977).

Only the following 8 species were considered in our calculations :

Family Microphallidae.

Maritrema subdolum (Jagerskiold, 1908).

Microphallus claviformis (Brandes, 1888).

+ 2 species whose cercariae encysted in the sporocyst without leaving the host :

Maritrema syntomocyclus Deblock and Tran Van Ky, 1966.

Microphallus somateriae (Kulatchkova, 1958).

As these species could only be determined unequivocally after artificial excystment, they are counted together.

Family Heterophyidae.

Aphalloides coelomicola Dollfus, Chabaud and Golvan, 1967.

Family Notocotylidae.

Unidentified cercaria, designated as Cercaria N.

Table I. Infection of *Hydrobia stagnorum* : number of infected snails and %.

Family	Species	Pre cercarial stage	1969-1970		1972-1974		1969-1974	
			nr	%	nr	%	nr	%
Microphallidae								
	<i>Maritrema subdolum</i>	Sporocyst	199	5.8	1864	14.4	2063	12.6
	<i>Microphallus claviformis</i>	"	39	1.1	629	4.9	668	4.1
	Microphallid cysts	"	124	3.6	725	5.6	849	5.2
Heterophyidae								
	<i>Aphalloides coelomicola</i>	Redia	128	3.8	716	5.5	844	5.2
Notocotylidae								
	Cercaria N	Redia	105	3.1	841	6.5	946	5.8
Echinostomatidae								
	<i>Himastha militaris</i>	Redia	12	0.4	148	1.1	160	1.0
Monorchidae								
	<i>Asymphylodora demeli</i>	Redia	537	15.8	61	0.5	598	3.7
Total number of snails examined :			3400		12923		16323	

Family Echinostomatidae.

Himasthla militaris (Rudolphi, 1802).

Family Monorchidae.

Asymphyloglora demeli Markowski, 1935.

The number of infected snails and % infection can be found in table I. The other 5 species occurred very rarely (less than 0.1 % infection).

Although the infection-rate of the different species showed changes in time (not only seasonally but also from year to year), the fluctuations followed the same pattern for almost all species and the proportions between the different infection-rates remained more or less the same. Only 1 species showed a marked difference in this respect before and after 1971, namely *A. demeli*. During the first period of the investigation, it was the dominant species with a mean infection-rate of 15.8 %. During the second period, this species had virtually disappeared (mean infection-rate 0.5 %). Combinations in which this species occurred are not included in the overall total of both periods taken together, as these results would be misleading.

The snails were measured and examined under a dissecting microscope; the shell was crushed and the tissues were lacerated with a pair of needles. The liberated cercariae were examined further under the microscope for identification.

Results

1. — Double infections.

We found 428 snails harbouring double infections, i.e. 2.6 % of the total number examined.

Table II gives the observed and expected number of double infections for all possible combinations, together with the χ^2 -values. The expected number was calculated using the formula $(a/N \times b/N)N$, where a and b are the number of snails infected with species a and b , and N the total number of snails examined. These figures can be found in table I.

The results can be grouped in 4 categories :

A. — Species exhibiting no marked antagonism (P mostly > 0.1).

M. subdolum + Cercaria N

M. subdolum + *A. demeli*

M. claviformis + *H. militaris*

M. claviformis + *A. demeli*

Microphallid cysts + Cercaria N

M. claviformis + *A. coelomicola*

» » + *H. militaris*

» » + *A. demeli*.

B. — Species exhibiting only a mild antagonism ($0.05 > P > 0.025$).

M. subdolum + *H. militaris*.

C. — Species exhibiting a strong antagonism ($P < 0.005$)

M. subdolum + *M. claviformis*

» » + Microphallid cysts

» » + *A. coelomicola*

M. claviformis + Microphallid cysts

» » + Cercaria N

» » + *A. coelomicola*

A. demeli + *A. coelomicola*

A. coelomicola + Cercaria N

D. — Species which were never found together

Cercaria N + *H. militaris*

» » + *A. demeli*

A. coelomicola + *H. militaris*

H. militaris + *A. demeli*.

2. — Triple infections.

Only 7 snails with a triple infection were found, all in the period 1972-74.

M. subdolum + Cercaria N + Microphallid cysts:

observed : 4

expected : 6.8

chi² : 1.15

M. subdolum + *A. coelomicola* + Microphallid cysts:

observed : 2

expected : 5.8

chi² : 2.49

M. subdolum + *M. claviformis* + Microphallid cysts:

observed : 1

expected : 5.1

chi² : 3.30.

Rather surprisingly, there is nowhere a significant difference between the observed and expected values. However, it has to be noted that the numbers are very small, and the χ^2 -test is only valid when the expected values are larger than 10 (Grémy and Salmon, 1969).

Discussion

As can be seen in table II, practically everywhere the observed frequencies of double infection are lower than the expected ones. However, in some cases these differences are not or hardly significant (categories A and B), in others they are extremely significant (categories C and D).

An important factor determining these differences seems to be the fact whether the cercariae develop in rediae or in sporocysts. From table II, it appears that:

1. double infections with both species forming rediae are extremely rare: only 2 combinations were found. The other 4 possible combinations were never observed (total number expected: 117; total number observed: 6);
2. when both species develop sporocysts, double infections were equally rare (total number expected: 226; total number observed: 60);
3. in mixed sporocyst-redia infections, there was far less discrepancy between expected and observed numbers: 8 combinations showed no significant difference with the expected values, 1 combination showed a difference on the 5 % level (not on the 2.5 % level), and only 3 combinations showed a marked deviation from the expected frequency.

It should be kept in mind that the observations are made on a naturally infected population, and that several factors can influence the probability of occurrence of two (or more) trematode species in the same host:

1. spatial distribution in the host: when both species occupy a different microhabitat within the host, competition will be minimal or absent. In our case, it seemed all species preferred the hepatopancreas as microhabitat. Only in very heavy infections, the gonad was invaded as well;
2. temporal distribution of the infective stages (miracidia). When two species have a clearly different seasonal cycle, the probability of double infection will be much lower than the theoretically calculated number. An analysis of the seasonal variations in infection of *H. stagnorum*, which will be published later, shows that the infection-rate of most trematode species follows the same temporal pattern (as we mentioned in the introduction *A. demeli* was a notable exception in this respect). In addition, we compared the observed and expected number of double infections for each quarter of the different years, and the total values per year and per quarter. As these figures fill about 8 pages, only one example is included (*M. claviformis* + *Cercaria* N, table III). The same pattern which emerges from the total values could be found in this more detailed analysis;

Table II. Double infection of *Hydrobia stagnorum* (O : observed — E : expected)

Species	1969-1970			1972-1974			Total		
	O	E	X ²	O	E	X ²	O	E	X ²
<i>M. subdolum</i> + <i>M. claviformis</i>	0	2.3	2.3	8	90.7	75.9	8	84.4	69.5
+ Microphallid cysts	5	7.3	0.7	45	104.6	34.2	50	107.3	30.8
+ <i>A. coelomicola</i>	3	7.5	2.7	35	103.3	45.5	38	106.7	44.5
+ Cercaria N	4	6.1	0.7	111	121.3	0.9	115	119.6	0.2
+ <i>H. militaris</i>	0	0.7	0.7	11	21.3	5.0	11	20.2	4.2
+ <i>A. demeli</i>	26	31.4	0.9	7	8.8	0.4			
<i>M. claviformis</i> + Microph. cysts	0	1.4	1.4	18	35.3	8.5	18	34.7	8.0
+ <i>A. coelomicola</i>	0	1.5	1.5	17	34.8	9.1	17	34.5	8.9
+ Cercaria N	1	1.2	0.03	21	40.9	9.7	22	38.7	7.2
+ <i>H. militaris</i>	0	0.1	0.1	4	7.2	1.4	4	6.5	1.0
+ <i>A. demeli</i>	4	6.2	0.8	4	3.8	0.3			
Microph. cysts + <i>A. coelomicola</i>	8	4.7	2.3	26	40.2	5.0	34	43.9	2.2
+ Cercaria N	2	3.8	0.8	40	47.2	1.1	42	49.2	1.1
+ <i>H. militaris</i>	1	0.4	0.9	6	8.3	0.6	7	8.3	0.2
+ <i>A. demeli</i>	11	19.6	3.8	4	3.4	0.1			
<i>A. coelomicola</i> + Cercaria N	0	4.0	4.0	1	46.6	44.8	1	48.9	47.1
+ <i>H. militaris</i>	0	0.4	0.4	0	8.2	8.2	0	8.3	8.3
+ <i>A. demeli</i>	5	20.2	11.6	0	3.4	3.4			
Cercaria N + <i>H. militaris</i>	0	0.4	0.4	0	9.6	9.6	0	9.3	9.3
+ <i>A. demeli</i>	0	16.6	16.7	0	4.0	4.0			
<i>H. militaris</i> + <i>A. demeli</i>	0	1.9	1.9	0	0.7	0.7			

Table III. Quarterly and yearly distribution of double infection of *Microphallus claviformis* and *Cercaria* N

	1972			1973			1974			1972-1974		
	O	E	X ²	O	E	X ²	O	E	X ²	O	E	X ²
I-III	0	0.2	0.2	4	6.7	1.1	0	0.7	0.7	4	6.0	0.7
IV-VI	0	1.1	1.1	2	4.4	1.3	1	1.4	0.1	3	6.5	1.9
VII-IX ...	4	14.7	9.4	2	4.8	1.6				6	18.6	8.6
X-XII	3	5.3	1.0	0	1.4	1.4				3	6.0	1.5
T	7	19.3	7.9	8	17.6	5.2	1	2.1	0.6	16	36.3	11.4

5. negative interaction between two species in the same microhabitat. The degree of this interaction will depend on the degree in which the niches of both species are overlapping. A general survey of these interactions between parasites is given by Halvorsen (1976). They can be divided into two major types:

A. direct antagonism: predation of one species on the other. Extensive experimental work has been done on the predatory activities of echinostome rediae, reviewed by Lim and Heyneman (1972); Lie *et al.* (1968) even suggested that this was a feature common to all rediae,

B. indirect antagonism: competition for common resources. According to Milne (1961), food and space are the principal resources for which competition arises. The mechanism of competition can be:

a interference, by which one species can limit or prevent the access to a resource to another species (most often through host-mediated interactions), or

b exploitation, by which a resource is more efficiently used by one of the two species.

Our results show that predation is certainly not a factor common to all rediae: in the large majority of mixed sporocyst-redia infections (9 out of 12) there is little or no negative interaction. Even the echinostome species, *H. militaris*, showed no evidence of negative interaction.

Only 3 sporocyst-redia combinations occurred less frequently than expected; in two of them, *A. coelomicola* was the redia-producing species. Here, predation is a possible explanation for the limited number of these combinations. The absence of negative interaction between *A. coelomicola* and the microphallid species with shortened life-cycle can be explained by the fact that the metacercarial cysts will make predation impossible.

The deviation from the expected frequency shown by the combination *M. claviformis* + *Cercaria* N is the result of a single marked discrepancy between expected and observed values in the third quarter of 1972, as can be seen in table III.

Negative interaction was always clearly apparent when both species possessed the same precercarial stage: sporocyst-sporocyst combinations occurred rarely, and redia-

redia combinations were practically never observed. Again, predation seems not to be the responsible factor: it is unlikely that rediae would only prey on other rediae, and not on sporocysts. In the case of sporocyst-sporocyst combinations, predation is obviously out of the question.

Therefore, we surmise that the simultaneous occurrence of 2 species in the same host is controlled by indirect antagonism, more precisely by competition for food.

When the feeding-mechanism of the precercarial stages is the same, there will be competition of the same type of food, and a more efficient exploitation of this resource will limit or prevent the presence or development of a second species. When the feeding mechanism is different in both species, there will be at most competition for space, and this seems not to be an important factor, as most sporocyst-redia combinations show no significant deviation from the expected frequencies.

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