FACTORS AFFECTING THE ACQUISITION OF *PLAGIORCHIS NOBLEI* (TREMATODA: PLAGIORCHIIDAE) CERCARIAE BY BLACK FLY (DIPTERA: SIMULIIDAE) LARVAE AND THE EFFECT OF METACERCARIAE ON HOST SURVIVAL

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ABSTRACT. Larvae of 4 species of black flies, *Prosimulium mixtum*, *Simulium vittatum*, *S. decorum* and *Stegopterna mutata*, were exposed in the laboratory to cercariae of the digenean *Plagiorchis noblei* in flowing water. Prevalence and intensity of infection of all species varied directly with exposure intensity and decreased with increasing water velocity; prevalence increased with larval size. Infection levels for *P. mixtum* were higher than for the 3 other species. Mortality among infected larvae of all species was 3 times that of controls. Exposure of black fly larvae to *P. noblei* cercariae may adversely affect their survival.

INTRODUCTION

In many parts of the world, not only do black flies rank as serious pests of animals and humans due to their annoying biting habits, but they also cause major economic, veterinary and medical problems as vectors of several parasitic diseases (Crosskey 1990). Conventional chemical control of black flies faces problems of environmental pollution, escalating costs and insecticide resistance (Guillet et al. 1980, Wallace and Hynes 1981) making the biological approach to control more attractive (Davidson and Sweeney 1983).

A wide range of naturally occurring and laboratory-reared parasites and pathogens are available as potential biological control agents of simuliids (Strand et al. 1977), but additional research into their propagation and feasibility for field use is still needed (Lacey and Undeen 1987).

Dempster et al. (1986), Rau et al. (1991) and Rau (1992) found that infections with the entomopathogenic cercariae of the digenean *Plagiorchis noblei* severely impair the development and survival of several mosquito species under laboratory and field conditions. The life cycle of these parasites characteristically involves an alteration of sexually and asexually reproducing generations. The entomopathogenic cercariae are produced by polyembryony within the tissues of the molluscan first intermediate host. *Plagiorchis noblei* cercariae emerge from the snail (*Stagnicola elodes*) each evening at dusk in response to decreasing light intensity (Webber et al. 1986). After a brief, free-swimming existence, cercariae penetrate the aquatic stages of a variety of insects to transform into metacercariae (Blankespoor 1977). Insects harboring metacercariae are ingested by an avian or mammalian definitive host, and develop into adult worms in the intestinal tract. Eggs are passed with the feces and are ingested by the snail host. Miracidia hatch in the intestinal tract of the snail and penetrate its tissues where developing sporocysts produce cercariae asexually.

The overall objective was to determine if the digenean *Plagiorchis noblei* may have potential as a biological control agent of black fly larvae. More specifically, the study establishes whether the entomopathogenic cercariae can infect black fly larvae in running water under controlled laboratory conditions, and whether abiotic factors, such as water velocity and exposure period, and biotic factors such as black fly species and larval size, can affect the level of parasite acquisition. Furthermore, the study addresses the spacial distribution of cercariae on the substrate under various conditions, and considers how such distributions may influence the prevalence and intensity of infection of the host population. Finally, the study establishes the effect of exposure and infection on the survival of black fly larvae.

MATERIALS AND METHODS

Sampling and identification of the parasite and the black fly hosts: Stagnicola elodes were collected in the spring of 1990 from a slowly flowing creek near Morgan Road, Baie d'Urfé, Québec and *Plagiorchis noblei*-infected individuals were maintained in the laboratory as described by Webber et al. (1986). *Plagiorchis noblei* was identified on the basis of egg morphometrics (Blankespoor 1974).

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Black fly larvae were collected between February and October 1990 from various small streams within a 90 km radius of Montréal, Québec. Larvae were identified using the keys of Wood et al. (1963) and Peterson (1970, 1981). The following species were exposed experimentally to cercariae: *Simulium vittatum*, *Prosimulium mixtum*, *Stegopterna mutata* and *Simulium decorum*.

Description of the experimental system and general test procedure: A closed pump-trough system based on the one described by Gaugler et al. (1980) was assembled. The system provides relatively uniform flow rates, excellent visibility and rapid water replacement. A pump (Aquaclear Power Head 800) recirculates 5 liters of aerated tap water (20 ± 2°C) from a 10 liter reservoir to the head of a sloping trough (35 × 19 × 7 cm). This creates a smooth sheet of water that flows through the trough at a rate of approximately 10 liters per minute.

Groups of approximately 120 field collected black fly larvae were introduced into each of 2 identical but independent troughs as described by Gaugler et al. (1980). The larvae were allowed 12 h to acclimate to the conditions before testing. Pre-soaked tropical fish food (TetraMin®, Staple Food, Tetra Co.) was provided *ad libitum* as a source of food for the larvae. Larvae that became detached from the substrate and were swept into the reservoirs prior to testing were removed. The mean number ± SD of larvae remaining in the 2 troughs (A and B) after 12 h was: A) 50 (±28) and B) 55 (±30). Approximately 90,000 freshly emerged cercariae from a pool of 14 *Stagnicola elodes* were introduced at the head of trough B. Cercariae were allowed to recirculate for a given period of time. Black fly larvae in trough A were not exposed to cercariae. Exposure to cercariae was terminated by flushing the system with 80 liters of fresh, aerated tap water. Fresh water was added to the reservoir and water from the trough was not allowed to recirculate during this period. Controls were treated identically. After flushing, water was again recirculated but "poly"-wool filters were introduced into the system to trap any remaining cercariae. Black fly larvae remained in the apparatus overnight for 12 hours.

Independent variables

a. Water velocity. Optimal water velocities differ for various black fly species. To determine the effect of water velocity on parasite acquisition, experiments were conducted at 5 different water velocities. Water velocities in the trough were adjusted by placing a stainless screen (mesh width 0.3 or 3 mm) at the downstream end of the trough and/or by increasing the slope of the trough. Water velocities were calculated by dividing discharge of the water by the width and depth of the sheet of the water in the trough. Water velocities of approximately 5, 11, 15, 23 and 40 cm per second were utilized.

b. Exposure period. Experiments were conducted over a range of 4 periods to determine the effect of exposure time on parasite acquisition. In a primary series of experiments, a 2 h exposure was used during which the suspension of cercariae was recirculated 240 times within the system. This period was chosen to simulate the period of natural release of cercariae from the snail host, which extends over approximately 2 hours at dusk (Webber et al. 1986). Tests were subsequently undertaken at much lower exposure periods: a 10 min period (which corresponds to 20 recirculations of the cercariae in the system), a 5 min period (which corresponds to 10 recirculations of the cercariae in the system), and a 5 min period but without recirculation. For each of the 5 water velocities, tests were done at each of the above 4 exposure periods.

c. Black fly species and larval size. To determine whether there were differences in parasite acquisition and host death among black fly species, four black fly species were exposed to cercariae. To ascertain if larval size effected parasite acquisition, larvae were grouped according to body length (<3.5 or ≥3.5 mm; maximum body length was 7 mm). However, simultaneous determination of the effect of larval size on parasite acquisition and host death was possible only in the case of one species, *S. decorum*, and then only in 10 experiments. The univoltine nature of *S. mixtum* and *St. mutata*, and other logistic limitations in the case of *S. vittatum*, made these species available over very short periods during the course of this study (Table 1). The mean number ± SD of exposed larvae <3.5 mm was 16 ± 11 and for exposed larvae ≥3.5 mm 50 ± 24. Corresponding numbers of controls were 15 ± 9 and 45 ± 22.

Dependent variables

a. Mortality, prevalence and intensity of infection. Twelve hours after exposure to cercariae, dead larvae (i.e., larvae not showing "avoidance reactions" as described by Chance[2]) were

[2] Chance, M. M. 1977. Influence of water flow and particle concentration on larvae of the black fly *Simulium vittatum* Zett. (Diptera: Simuliidae), with emphasis on larval filter-feeding. Ph. D. dissertation. University of Alberta, Edmonton.

Table 1. Experimental design: combinations of abiotic factors (5 water velocities and 4 exposure periods) and biotic factors (4 black fly species and 2 larval sizes) under which black fly larvae were exposed to *Plagiorchis noblei* cercariae in a closed pump-trough system.

Water velocity (cm/sec)	Exposure period (in terms of frequency of water recirculation)			
	1×	10×	20×	240×
5	S.d.(58)* S.d.s(7) St.m.(40)	S.d.(47)	S.d.(93) S.d.s(11)	P.m.(41,56[1]) S.v.(33)
11	S.d.(47) S.d.s(18) St.m.(33)	S.d.(67)	S.d.(106) S.d.s(14)	P.m.(38,44) S.v.(39)
15	S.d.(53) S.d.s(43) St.m.(19) P.m.(21)	S.d.(50)	S.d.(99) S.d.s(10) P.m.(25)	P.m.(35,51) S.v.(34)
23	S.d.(52) S.d.s(9) St.m.(20)	S.d.(76)	S.d.(106) S.d.s(18)	
40	S.d.(72) S.d.s(22) St.m.(26) S.v.(43)	S.d.(61)	S.d.(69) S.d.s(6)	P.m.(29) S.v.(19)

S.d. = *S. decorum* larvae ≥ 3.5 mm.
S.d.s = *S. decorum* larvae < 3.5 mm.
P.m. = *P. mixtum* larvae ≥ 3.5 mm.
St.t. = *St. mutata* larvae ≥ 3.5 mm.
S.v. = *S. vittatum* larvae ≥ 3.5 mm.
()* = number of larvae exposed.
[1] = replicate.

removed from the trough, lightly crushed under a coverslip, and examined for metacercariae using a compound microscope (40×). Prevalence (i.e., proportion of infected larvae) and intensity of infection (i.e., number of metacercariae per infected larva) were determined. Larvae that were still alive 12 h after exposure to cercariae were transferred to a holding container filled with 0.5 liters of aerated tap water and furnished with a magnetic stirrer. Larvae were maintained in an incubator at 20°C. Over the subsequent 8 days, dead larvae and pupae were removed daily and examined for metacercariae. At the end of 8 days all remaining larvae were removed, crushed and examined as described above. For statistical analysis, only mortality on days 1 and 4 post-exposure was considered.

b. Parasite distribution in the experimental system. To assess the distribution of the parasite within the apparatus and to determine the degree of contact between *P. mixtum* larvae and *P. noblei* cercariae over the 2-h exposure period, 2 ml water samples were taken at 10 min intervals at 3 different sites in the apparatus: a) from the sheet of water in the trough, b) from the bottom surface of the trough, and c) from the bottom of the reservoir. Samples from the bottom of the trough and the reservoir were obtained by scraping the surface with the tip of a pipette. The number of cercariae per sample was determined using a dissecting microscope.

Statistical analysis: A multiple regression analysis was used within the concept of the generalized linear models (G.L.M.) to assess the predictive value and impact of each of the independent on the dependent variables. This technique is adapted to data with binomial and/or Poisson distributions, and compensates for an unbalanced experimental design (Armitage and Berry 1987). The analysis was performed with the most recent version of multivariate general linear hypothesis of the software package Systat (Wilkinson 1990) on an IBM personal computer. Linearity between the dependent variables and the independent variable water velocity was obtained by transforming the values of water velocity to their common logarithms.

To verify relationships between the 4 independent variables, Pearson correlation coefficients were calculated. Spearman coefficients of correlation were calculated between intensity of infection and the mortality of infected larvae. The Kruskal-Wallis procedure was applied to test for the statistical significance of differences

in mortality between exposed larvae and controls. A chi-square procedure tested the statistical significance of the differences between parasite distribution in the system. An alpha of 0.05 was used as the level of significance for all statistical analyses (Sokal and Rohlf 1981).

RESULTS

Prevalence of infection: Metacercariae were found in the haemocoel of the black fly larvae; no cercariae or metacercariae were seen in the digestive tract. The prevalence of infection in the trough system ranged from 0 to 84% with a mean of 27% (Table 2). Prevalences higher than 50% were found almost exclusively among *P. mixtum* larvae. Prevalences lower than 50% were found in *S. decorum*, *S. vittatum* and *St. mutata*. Exposure of large *S. decorum* larvae (≥3.5 mm) to *P. noblei* cercariae at water velocities ≥15 cm/sec resulted in a mean prevalence of infection of 28%. In contrast small larvae were never infected. The prevalence of infection among larvae of all 4 species was generally highest at the lowest water velocity (5 cm/sec). Prevalence of infection among large *S. decorum* and *S. vittatum* larvae exposed to the parasite for longer periods (10, 20 and 240 recirculations) was generally more than 50% higher than when they were exposed only once for a short period of time (Table 2).

Because the independent variables "exposure period" and "black fly species" were highly correlated (r = 0.78, $P = 0.0001$), the following G.L.M. statements were used:

1° prevalence = constant + log water velocity + larval size + black fly species

2° prevalence = constant + log water velocity + larval size + exposure period

Both models indicated that the prevalence of infection among small larvae was significantly lower than that of large larvae ($P < 0.05$). Furthermore, the prevalence of infection decreased significantly ($P < 0.05$) with increasing water velocity. The first model ($R^2 = 0.661$) also revealed that the prevalence of infection differed significantly among species ($P < 0.01$). The prevalence of infection among *P. mixtum* was higher than among the three other species. The second model ($R^2 = 0.462$) revealed that the prevalence of infection was significantly greater for larvae of all 4 species when they were exposed more than once to the parasite ($P < 0.05$).

Intensity of infection: The intensity of infection ranged from 1 to 25 metacercariae per larva with a mean of 5 (Table 3). Intensities of more than 7 metacercariae per larva were found in *P. mixtum* larvae, or in *S. decorum* and *S. vittatum* larvae exposed to the parasites at the lowest water velocity (5 cm/sec). In general, the intensity of infection was highest for all 4 species at the lowest water velocity. The intensity of infection among small *S. decorum* larvae was generally less than half of the intensity among large larvae when both were exposed to cercariae 20

Table 2. Prevalence of infection (%) with *Plagiorchis noblei* metacercariae in black fly larvae after exposure to *Plagiorchis noblei* cercariae in a closed pump-trough system.

Black fly species/Larval size/Exposure period[1]	Water velocity (cm/sec)				
	5	11	15	23	40
S.d./s/1×	29	0	0	0	0
S.d./l/1×	10	13	6	21	16
St.m./l/1×	35	6	26	10	15
P.m./l/1×			52		
S.v./l/1×					6
S.d./l/10×	40	25	35	23	26
S.d./s/20×	27	14	0	0	0
S.d./l/20×	42	33	15	19	26
P.m./l/20×			84		
P.m./l/240×	80	68	54		78
P.m./l/240×	36	73	22		
S.v./l/240×	67	10	15		21

S.d. = *S. decorum*.
St.m. = *St. mutata*.
P.m. = *P. mixtum*.
S.v. = *P. vittatum*.
s = larvae < 3.5 mm.
l = larvae ≥ 3.5 mm.
[1] = in terms of frequency of water recirculation.

Table 3. Intensity of infection with *Plagiorchis noblei* metacercariae (±SE) in viable black fly larvae after exposure to *Plagiorchis noblei* cercariae in a closed pump-trough system.

Black fly species/Larval size/Exposure period[1]	Water velocity (cm/sec)				
	5	11	15	23	40
S.d./s/1×	3(± 0)	—	—	—	—
S.d./l/1×	3(± 1)	5(± 2)	1(± 0.5)	3(± 1)	4(± 1)
St.m./l/1×	5(± 2)	3(± 2)	2(± 0.2)	1(± 0)	1(± 0.3)
P.m./l/1×			2(± 0.3)		
S.v./l/1×					3(± 1)
S.d./l/10×	8(± 2)	7(± 2)	2(± 1)	3(± 1)	3(± 0.4)
S.d./s/20×	5(± 3)	1(± 0)	—	—	—
S.d./l/20×	12(± 2)	5(± 1)	2(± 0.1)	6(± 2)	3(± 0.5)
P.m./l/20×			4(± 1)		
P.m./l/240×	25(± 5)	4(± 1)	7(± 2)		4(± 0.5)
P.m./l/240×	16(± 3)	9(± 2)	10(± 4)		
S.v./l/240×	8(± 3)	4(± 2)	3(± 0.5)		6(± 2)

S.d. = *S. decorum.*
St.m. = *St. mutata.*
P.m. = *P. mixtum.*
S.v. = *S. vittatum.*
s = larvae < 3.5 mm.
l = larvae ≥ 3.5 mm.
[1] = in terms of frequency of water recirculation.
— = tests where the prevalence was 0.

times. Similarly, higher intensities were found in *S. decorum* and *P. mixtum* larvae exposed to the parasites more than once (Table 3).

Again, because of strong correlation between variables, the following G.L.M. statements were used to predict intensity of infection:

1° intensity = constant + log water velocity + larval size + black fly species

2° intensity = constant + log water velocity + larval size + exposure period

Both models revealed that the intensity of infection decreased significantly ($P = 0.001$) with increasing water velocity. Furthermore, the first model ($R^2 = 0.479$) showed that the intensity of infection differed significantly among species ($P < 0.05$). More metacercariae per larva were found in *P. mixtum* larvae than in *St. mutata* larvae. The second model ($R^2 = 0.497$) revealed that the intensity of infection rose significantly ($P < 0.05$) with an increase in exposure period. More metacercariae per larva were found when these were exposed to the parasites 240 times rather than once.

Mortality

a. 1 day post-exposure. Mortality of infected larvae 1 day post-exposure ranged from 0 to 100% with a mean of 29%. Mortality greater than 50% was found only among larvae exposed at water velocities < 23 cm/sec. All small infected larvae (*S. decorum*) died 1 day post-exposure (Table 4).

Because of correlations between variables, the following G.L.M. statements were used to predict mortality of infected larvae 1 day post-exposure:

1° mortality = constant + log water velocity + larval size + black fly species

2° mortality = constant + log water velocity + larval size + exposure period

Both models revealed that, in general, mortality of infected larvae 1 day post-exposure decreased significantly with an increase in water velocity ($P < 0.001$), and that mortality among large infected larvae was significantly lower than among small larvae ($P < 0.001$). Furthermore, the first model ($R^2 = 0.678$) showed no significant difference in mortality of infected larvae among species, and the second model ($R^2 = 0.675$) revealed that exposure period had no significant impact on mortality of infected larvae. There was also a significant correlation between mortality of infected larvae 1 day post-exposure and intensity of infection ($r = 0.604$; $P < 0.05$).

The mortality of exposed but uninfected larvae ranged from 0 to 62% with a mean of 8%. The mortality of the unexposed control larvae ranged from 0 to 45% with a mean of 10% (Table 4). No significant difference in mortality was found between these two groups of uninfected

Table 4. Mortality (%) of black fly larvae 1 day and 4 days post-exposure to *Plagiorchis noblei* cercariae in a closed pump-trough system.

Black fly species/Larval size/Exposure period[1]	Water velocity (cm/sec)				
	5	11	15	23	40
S.d./s/1×	100[2];100[3]	—	—	—	—
	0[4];0[5]/8[6];8[7]	0;0/0;0	0;0/0;0	0;0/0;0	0;0/0;0
S.d./l/1×	33;100	0;83	0;67	9;64	0;67
	4;8/6;13	0;2/0;20	0;18/0;2	2;22/0;7	0;31/0;6
St.m./l/1×	71;86	0;100	40;80	0;50	0;50
	19;69/41;74	10;39/42;85	14;50/6;63	17;50/36;79	9;82/13;88
P.m./l/1×			9;—		
			10;—/—		
S.v./l/1×					0;—
					0;—/—
S.d./l/10×	58;95	65;88	18;65	24;53	0;44
	4;75/13;28	4;30/7;53	10;39/10;55	10;14/8;22	0;16/3;47
S.d./s/20×	100;100	100;100	—	—	—
	38;38/33;33	62;62/33;33	10;10/0;0	0;0/0;0	0;0/0;0
S.d./l/20×	54;90	43;74	0;13	5;25	44;78
	15;37/19;49	4;39/11;37	2;8/0;5	2;17/5;9	4;29/1;16
P.m./l/20×			5;—		
			0;—/—		
P.m./l/240×	45;—	4;—	58;—		20;—
	13;—/16;—	0;—/3;—	13;—/45;—		23;—/8;—
P.m./l/240×	65;—	38;—	27;—		
	6;—/12;—	42;—/20;—	13;—/5;—		
S.v./l/240×	23;—	0;—	0;—		0;—
	0;—/9;—	0;—/0;—	0;—/2;—		0;—/14;—

[1] = in terms of frequency of water recirculation.
[2] = infected larvae 1 day post-exposure.
[3] = infected larvae 4 days post-exposure.
[4] = uninfected larvae 1 day post-exposure.
[5] = uninfected larvae 4 days post-exposure.
[6] = unexposed larvae 1 day post-exposure.
[7] = unexposed larvae 4 days post-exposure.
— = tests where the prevalence was 0 or where no data were collected.
S.d. = *S. decorum.*
St.m. = *St. mutata.*
P.m. = *P. mixtum.*
S.v. = *S. vittatum.*
s = larvae < 3.5 mm.
l = larvae ≥ 3.5 mm.

larvae. A highly significant difference in mortality ($U = 1086.5$; $P = 0.008$) was found between such uninfected larvae and infected larvae 1 day post-exposure.

The same G.L.M. models used to predict mortality among infected larvae were used for uninfected larvae. Both models showed that among uninfected larvae, mortality also decreased significantly with an increase in water velocity ($P < 0.05$). One of the models ($R^2 = 0.421$) demonstrated that there were significant differences in mortality among uninfected larvae of the 4 species ($P < 0.01$). The models also showed that the independent variables "larval size" and "exposure period" had no significant impact on the mortality of exposed but uninfected larvae.

b. 4 days post-exposure. The cumulative mortality of infected larvae 4 days post-exposure ranged from 0 to 100% with a mean of 73%. Most infected larvae that had not died after 4 days had pupated. Mortalities greater than 80% were found only among larvae exposed at water velocities of <23 cm/sec (Table 4).

The same G.L.M. statements used above for mortality 1 day post-exposure were used for mortality 4 days post-exposure. Both models showed that the mortality of infected larvae 4 days post-exposure decreased significantly with an increase in water velocity ($P < 0.01$), and that mortality among small infected larvae was not significantly lower than among large larvae. In addition, the first model ($R^2 = 0.489$) showed

no significant difference in mortality of infected larvae among species, and the second model (R^2 = 0.540) revealed that exposure period had no significant impact on the mortality of infected larvae. A significant correlation between mortality of infected larvae 4 days post-exposure and intensity of infection (r = 0.684; $P < 0.05$) was also found.

The mortality of exposed but uninfected larvae ranged from 0 to 82% with a mean of 26%. The mortality of the unexposed control larvae ranged from 0 to 88% with an mean of 28% (Table 4). No significant difference in day 4 post-exposure mortality was found between these 2 groups of uninfected larvae. However, mortality among infected larvae was significantly higher (U = 621; $P < 0.001$) than among their uninfected counterparts.

The same G.L.M. models used for infected larvae were used to predict mortality among uninfected larvae. Both models showed that there was no significant decrease in mortality with increased water velocity, and that the mortality among small larvae was significantly lower than among large larvae ($P < 0.05$). The first model ($R^2 = 0.727$) also demonstrated that mortality among uninfected *S. decorum* larvae was significantly lower than among uninfected *St. mutata* larvae ($P < 0.001$). The second model ($R^2 = 0.297$) showed that exposure period had no significant impact on the mortality of uninfected larvae.

Parasite distribution in the experimental system: At water velocities of 5 cm/sec, a mean ± SE of 4.0 ± 0.6 cercariae was found in samples of water flowing through the trough. Samples scraped from the bottom of the reservoir or the trough yielded 5.0 ± 0.8 and 44.3 ± 5.6 cercariae, respectively. In contrast, at water velocities of 40 cm/sec most cercariae were found at the bottom of the reservoir (88.3 ± 3.9 cercariae per sample), and fewer cercariae (4.0 ± 0.7) were recovered from the bottom of the trough. Again, 4.0 ± 0.6 cercariae were recovered from the water flowing through the trough.

This difference in distribution at low and high water velocities was highly significant ($P < 0.001$; $\chi^2 = 103.5$). The distributions of the parasites at water velocities of 11 and 15 cm/sec were similar to those at velocities of 40 cm/sec. The mean numbers ± SE of parasites per sample were 4.0 ± 0.5 and 2.0 ± 0.4, respectively, per sample taken from water flowing through the trough, 10.0 ± 0.8 and 5.0 ± 0.4 parasites, respectively, in samples scraped from the bottom of the trough, and 49.0 ± 4.1 and 37.4 ± 7.8 parasites in samples taken from the bottom of the reservoir.

DISCUSSION

The data suggest that *P. noblei* cercariae can penetrate the cuticle of black fly larvae in rapidly flowing water, a feat accomplished by only a few species of parasites, among them the mermithid *Neomesomermis flumenalis* (Molloy and Jamnback 1975). Although the actual process of penetration was not observed, the presence of encysted metacercariae in the haemocoel, and the absence of parasites from the digestive tract suggests that the external route of entry is the most common and that, unlike *Neoplectana carpocapsae*, they are not ingested by the black fly larvae (Gaugler and Molloy 1981). Contact between the host and the parasites seemed to be enhanced by strands of silk secreted by the black fly larvae.[3]

Prevalence and intensity of infection differed significantly among species: the highest prevalences (>50%) and intensities of infection (>7 metacercariae per larva) were found primarily among *P. mixtum* larvae, suggesting that this species is more susceptible to *P. noblei* infections than are *S. vittatum*, *S. decorum* and *St. mutata*. The observed differences in susceptibility in the present trials may be attributed to water temperature. Thus, Edman and Simmons (1985) stated that *Prosimulium*, *Cnephia* and *Stegopterna* species do best at water temperatures of 5–10°C, whereas *Simulium* species develop well at temperatures of 20 ± 2°C. The water temperatures used in the present trials (20 ± 2°C) may thus have favored the development of *Simulium* species and impaired the development, and concomitantly, the resistance of the *P. mixtum* larvae to parasite exposure or infection. In contrast, *St. mutata* larvae, which were taken together with *P. mixtum* from water at 9–12°C, did not seem to be affected by the elevated water temperatures. Thus, elevated water temperatures may weaken resistance to parasite exposure/infection of one (*P. mixtum*) but not all "cold" water species tested. Differences in susceptibility between various black fly species may also be attributed to other secondary factors such as differences in avoidance reactions and subsequent grooming responses to dislodge the parasites.

Prevalence, but not the intensity, of infection was significantly lower among small than among large larvae of *S. decorum*. In contrast, conventional insecticides and growth regulating substances are generally most effective against the

[3] Jacobs, P. 1991. *Plagiorchis noblei* and blackfly larvae: factors affecting parasite acquisition and the effect of infection on host survival. M. Sc. dissertation. McGill University, Montréal.

younger, smaller larvae (Gjullin et al. 1950, Rodrigues et al. 1983). The same holds true for *B.t.i.* (Molloy et al. 1981) and mermithid nematodes (Molloy and Jamnback 1975). A similar increase in susceptibility to *P. noblei* infections with increasing body size of a mosquito host [*Aedes aegypti* (Linn.)] was reported by Dempster and Rau (1987) and by Gaugler and Molloy (1981) for the nematode *N. carpocapsae* in black fly larvae. A greater body surface may enhance contact with cercariae and result in an increase in the prevalence and intensity of infection among larger larvae. Behavioral differences between small and large larvae may also influence the level of infection. They may actively avoid the relatively large cercariae, whereas large larvae may not. Furthermore, small larvae may produce less silk, which may decrease contact with the parasite. Small larvae may also be unable to retain parasites within their cephalic fans as described by Molloy and Jamnback (1975) for first and second instars of *S. vittatum* exposed to mermithid preparasites. Black flies exhibit the negative exponential mortality curve typical of insects (Cummins 1987). Thus, the probability of dying is greatest in the early stages and decreases with age. This may enhance the control potential of *P. noblei*; cercariae may act after density-dependent mortality factors have taken their toll.

With increasing water velocity (and concomitant reduction in water depth) there is a significant decline in the prevalence and intensity of infection. This may be due because cercariae attached to the larvae are more easily swept away by the force of the water at high velocities.[3] The highest prevalences and intensities of infection were consistently found at the lowest water velocity (5 cm/sec) whereas levels of infection at the 4 higher water velocities were more comparable to each other. Only at the lowest water velocity did significantly more parasites accumulate on the bottom of the trough of the recirculating system. Consequently, more parasites may have come into contact with the host, leading to a higher prevalence and intensity of infection. Not only may cercariae be able to adhere to the surface of the trough directly, but enhanced production and/or persistence of silk threads and pads may further facilitate adhesion and thus contact with the target insect.[3]

Prevalence and intensity of infection rose significantly with duration of exposure of larvae to cercariae (i.e., as larvae were exposed to larger numbers of parasites). A similar increase in the prevalence of infection and a concomitant increase in mortality accompanied a rise in the concentration (dose) of chemicals (Rodrigues et al. 1983) and *B.t.i.* (Lacey and Undeen 1984). However, at high water velocities most parasites are quickly removed from contact with their targets, and thus chances of parasite transmission are perhaps lower than expected. Nevertheless, enough parasites could recirculate to account for the higher levels of infection that accompanied longer exposure periods.

Three times as many infected as uninfected larvae had died one day and 4 days post-exposure. Furthermore, there was a significant positive correlation between intensity of infection and mortality on both days. This suggests that *P. noblei* infections severely impair the survival of black fly larvae. A similar effect of *P. noblei* infections on the survival of *Ae. aegypti* larvae was found by Dempster et al. (1986).

Although the intensity of infection was significantly higher in *P. mixtum* larvae than in the other species, mortality was not. This may either have a purely statistical basis (i.e., intensity of infection may account only for a fraction of the generally random event "mortality") or *P. mixtum* may be more resistant to the effects of *P. noblei* infection. Mortality among uninfected *St. mutata* larvae was significantly higher than among the other species. Nevertheless, this "weakness" did not render them more susceptible to infection.

Mortality of infected larvae decreased significantly with increasing water velocity, possibly since the intensity of infection was significantly lower at higher water velocities. However, mortality among uninfected larvae also decreased significantly with increasing water velocity 1 day, but not 4 days post-exposure. This suggests that the significantly enhanced mortality among infected larvae at low water velocities was due to a combination of higher intensity of infection and the general adverse effects of low water velocities on the survival of the larvae. There are no such effects 4 days post-exposure, since all larvae had been transferred by this time from the recirculation pump to a magnetic stirrer system, which generated higher water velocities. The adverse influence of low water velocities, even on uninfected larvae, is not surprising because all larvae were taken from habitats with water velocities in excess of 40 cm/sec.

Larval size affected mortality. Small larvae invariably died 1 day after infection whereas only 22% of the large, infected larvae did so. Similarly, more than 66% of large, infected larvae died or had pupated 4 days post-exposure. In contrast, uninfected small larvae had a significantly lower mortality than large larvae 4 days post-exposure. This suggests that small larvae survive better than large larvae when uninfected, but once infected, small larvae die quickly.

In summary, as a model system, *P. noblei* has provided considerable insight into how entomopathogenic digeneans may interact with their targets and possibly effect some control. *Plagiorchis noblei* cercariae certainly do not satisfy the criteria of the ideal larvicide (Kurtak et al. 1987). Nevertheless, the study suggests that the parasite may exert control on blackfly larvae under certain conditions: the velocity of the water must be low, repeated exposure or high doses are needed, and the target population must consist of relatively large larvae of a susceptible species. Furthermore, low water temperatures may severely curtail parasite transmission.

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