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Primary Research Paper

Distribution and seasonal abundance of trematode parasites (Trematoda: Allocreadiidae: *Crepidostomum* spp.) in burrowing-mayfly nymphs (Ephemeroptera: Ephemeridae: *Hexagenia* spp.) from connecting rivers of the Laurentian Great Lakes

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Abstract

Burrowing-mayfly nymphs such as *Hexagenia* spp. have been used extensively in North America and Europe as a biomonitoring tool to indicate mesotrophic water quality, yet infestation by associated parasites has not been well documented. We performed laboratory analysis of archived samples of *Hexagenia* spp. nymphs collected in 1985 and 1986 to provide base-line data on the distribution (1985) and seasonal infestation (1986) of the trematode parasite *Crepidostomum* spp. in *Hexagenia* spp. nymphs in connecting rivers between Lakes Superior and Erie of the Laurentian Great Lakes. In May and June 1985, frequency of occurrence of metacercariae was widely distributed throughout the connecting rivers (63% of 203 stations with nymphs), except in areas where nymph densities were relatively low (i.e., ≤69 nymphs/m^2). Distribution was probably underestimated in the present study because of low probability (mean = 31%, range = 0–57%) of detecting infestation in a small number of collected nymphs (≤10) at nymph densities ≤69/m^2. In 1986, seasonal infestation between April and October occurred in 3.3% (627) of 18696 nymphs. Overall prevalence, mean intensity, and mean abundance of parasites at one station in the St. Marys River indicate parasite transmission occurred between June and September. This period of transmission is dependent on the life-cycle of the parasite. In addition, the life-cycle of *Hexagenia* spp. determines which annual cohort of nymphs is infested and therefore, the duration of infestation. Although, no impacts of infestation on *Hexagenia* spp. nymphs were observed in the present study, infestation intensities were high enough (≥25 metacercariae per nymph) at one station in the St. Marys River to potentially cause tissue damage in a high proportion (53%) of infested nymphs.

Introduction

Burrowing-mayfly nymphs (Ephemeroptera: Ephemeridae: e.g., *Hexagenia* spp.) have been extensively studied in North America and Europe because they are easily sampled and identified, and their absence is an excellent indicator of excessive organic pollution (Britt, 1955; Fremling, 1973; Cook & Johnson, 1974; Fremling & Johnson 1990; Bij de Vaate et al., 1992; Schloesser et al., 2000). The presence of burrowing-mayfly nymphs is valued and often recognized in management plans that define beneficial uses of water for the general public including fishing and water quality (Cochran, 1992; Krieger et al., 1996; Ohio Lake Erie Commission, 1998; Schloesser & Nalepa, 2001). However, parasites of burrowing mayflies have not been studied nearly as extensively as burrowing mayfly nymphs, especially in large water systems such as the Laurentian Great Lakes (Hunt, 1953; Fremling, 1960; Ericksen, 1963; Hudson & Swanson, 1972; Horst, 1976; Schloesser et al., 2000). To date, only parasites in fish over a wide geographic area have been studied in the Great Lakes, and possibly elsewhere (Nepszy, 1988). Lack of studies examining parasites in mayfly nymphs is attributed to infrequency of parasitism, taxonomic uncertainties, and life-cycle complexities of individual parasites and hosts.

This base-line study of *Crepidostomum* spp. in burrowing mayflies was initiated because *Hexagenia* spp. nymphs are an important environmental indicator, little is known about the distribution and relative abundance of its parasites, and it is possible *Crepidostomun* spp. may affect the value of *Hexagenia* spp. and other burrowing mayfly species as biomonitoring tools. To these ends, we examined archived samples containing *Hexagenia* spp. nymphs for the presence of *Crepidostomum* spp. parasites (Edsall et al., 1991; Schloesser et al., 1991). Archived samples were collected in 1985 and 1986 throughout connecting rivers between Lakes Superior, Huron, and Erie of the Laurentian Great Lakes.

Methods

We examined archived samples of *Hexagenia* spp. nymphs collected at 203 stations in May and June 1985 by Schloesser et al. (1991) to assess the frequency of occurrence of *Crepidostomum* spp. in nymphs throughout connecting rivers between Lakes Superior, Huron, and Erie of the Laurentian Great Lakes. In addition, we examined samples from 11 stations collected monthly April to June and August to October 1986 by Edsall et al. (1991) to assess seasonal infestation values in the same connecting rivers.

Methods follow Edsall et al. (1991) and Schloesser et al. (1991) and briefly include: in 1985, three replicate samples were obtained at each station; in 1986, 15 replicate samples were obtained each month at each of 11 stations. Sediments containing nymphs were collected with a standard (0.048 m^2 jaw opening) Ponar grab, washed over a 0.595-mm mesh sieve to remove fine sediment, and individually preserved in a 10% formalin-phloxine B dye solution (Schloesser et al., 1991). In the laboratory, residue from each sample was placed in an enamel pan and nymphs were removed and stored in 5% formalin.

In the present study, 2% glycerin was added to archived samples for approximately six months before examination to reduce stiffness of nymph and parasite tissues. Cysts were identified and counted at 7–10× magnification based on cyst appearence as orange to brown spheres ranging in diameter from 0.15 to 0.25 mm (mean ± SE = 0.20 ± 0.002 mm, n = 200) (Fig. 1). In 1985, metacercariae were classified as present at a station if 1 nymph was found to contain a cyst. Metacercariae were classified as absent at a station when all collected nymphs at a station were examined and found to contain no cysts. In 1986, numbers of cysts in individual nymphs were enumerated and lengths of individual nymphs (from rostrum to end of abdomen) were determined.

Identification of cysts was based on examination of metacercariae (1–5/nymph) dissected from nymphs (5–10/sampling) obtained at 15 stations (4 randomly selected stations in each river and 3 stations in Lake St. Clair) in 1985, and all 11 stations where cysts were found in May and September 1986, and station 51 each sampling period in 1986. Dissected metacercariae were (one per cyst) examined at 600× magnification and identified to the genus *Crepidostomum* (Trematoda: Allocreadiidae) based on size and color of cysts (above), a terminal oral sucker, 6 oral papillae, and 2 testes (Hopkins, 1934). Species identification was not possible because this requires the definitive host stage of *Crepidostomum* spp. only found in teleost fishes (Hopkins, 1934). However, based on size and color of cysts, generic identification, and general morphology of metacercariae, there is a high probability that metacercariae in the present study were *Crepidostomum cooperi* and/or *C. (Megalognia) ictaluri* (G. Esch & A. Choudhury, acknowledgements, personal communication). In addition, these species are among the most frequently found in fishes from tributaries and lakes of connecting rivers included in the present study, especially waters adjacent to and in the St. Marys River (Hopkins, 1934; Cooper et al., 1977; Muzzall, 1982; 1984; 1986; Caira, 1985; Muzzall et al., 1987). Consequently, *Crepidostomum* was only

Figure 1. Encapsulated cysts (i.e., small spheres) containing trematode parasites (*Crepidostomum* spp.) found in burrowing mayfly nymphs (*Hexagenia* spp.) collected throughout connecting rivers between Lakes Superior, Huron, and Erie of the Laurentian Great Lakes. Within each cyst is one metacercaria of *Crepidostomum* spp.

identified to genus and data were pooled for analysis. Examination of exterior surfaces of nymphs and interior body structures from dissected nymphs (n = 100 nymphs) collected throughout the study area (n = 20 sites) revealed no differences between the number of cysts observed before and after dissection. Therefore, enumeration of cysts based on examination of the surface of nymphs represented total infestation values.

Burrowing mayfly nymphs were also identified to genus (*Hexagenia*) because two species of this taxon (*H. limbata* and *H. rigida*) co-occur in waters of the Great Lakes and it is not possible to separate species based on nymph morphology (Schloesser & Hiltunen, 1984; Krieger et al., 1996; Schloesser et al., 2000). These two species can only be distinguished based on adult characteristics once nymphs emerge from the water. In the St. Marys River, the majority are believed to be *H. limbata* (Schloesser & Hiltunen, 1984; Edsall et al., 1991), whereas in the St.Clair-Detroit River system both species are probably present (possibly 75% *H. limbata* and 25% *H. rigida*) (Ciborowski & Corkum, 1988; Krieger et al., 1996; Corkum et al., 1997; Schloesser & Nalepa, 2001). Similarities of growth, reproduction, and life-histories of the two species of nymphs has resulted in their being treated as one taxon in the Great Lakes (Hiltunen & Schloesser, 1983; Ciborowski & Corkum, 1988; Reynoldson et al., 1989; Corkum et al., 1997; Schloesser & Nalepa, 2001). Similar to *Crepidostomum* spp., the two species of *Hexagenia* in the present study were pooled for analysis.

Infestation values of prevalence (number of infected nymphs per examined nymph expressed as a percent), intensity (number of parasites per infected nymph), and abundance (number of parasites per examined nymph) were used to assess infestation of nymphs by metacercariae in 1986 (Bush et al., 1997). Statistical differences ($p < 0.05$) were determined using χ^2 analysis (number of stations with and without metacercariae and numbers of infested nymphs (i.e., prevalence)) and Mann–Whitney *U*-tests with adjusted degrees of freedom for unplanned comparisons (intensity and abundance). Pearson product-moment linear correlation was used to assess relationships between length, intensity, and mean abundance of nymphs (Sokal & Rohlf, 1995).

Results

Distribution

In 1985, examination of samples of *Hexagenia* spp. nymphs indicate that metacercariae of *Crepidostomum* spp. were found throughout the study area (Fig. 2). Nymphs occurred at 81% (203) of the 250 stations and metacercariae occurred at 63% (128) of the 203 stations where nymphs were present (Table 1). Nymphs occurred at about the same proportion of stations in the St. Marys, St. Clair, and Detroit rivers and Lake St. Clair (72–88% of stations). However, the St. Clair and Detroit rivers had lower numbers of stations with metacercariae present ($p < 0.05$, 6 and 11, respectively) than the St. Marys River (90) and Lake St. Clair (21). Metacercariae did not occur in large portions of the St. Marys River north of station 30, in the St. Clair River between Lakes Huron and St. Clair, along the perimeter of Lake St. Clair, and in the upper and lower Detroit River (Fig. 2).

In general, occurrence of metacercariae at individual stations was related to the number of nymphs examined at each station (Table 2). Examination of 1–10 nymphs (i.e., 7–69 nymphs/m^2) resulted in low probabilities (0–57%) of finding infestation, whereas examination of 11 or more nymphs (≥75 nymphs/m^2) resulted in high probabilities (81–100%) of finding infestation. Overall, only 31% of stations that had low numbers of nymphs (i.e., between 1 and 10) exhibited infestation. Higher proportions of stations in the St. Clair and Detroit rivers (68%) had stations with 1–10 nymphs than stations in the St. Marys River and Lake St. Clair (25 and 29%, respectively) (Table 1).

Seasonal abundance

In 1986, examination of 18696 *Hexagenia* spp. nymphs collected at 11 stations reveal that 627 (3.3%) nymphs contained a total of 14099

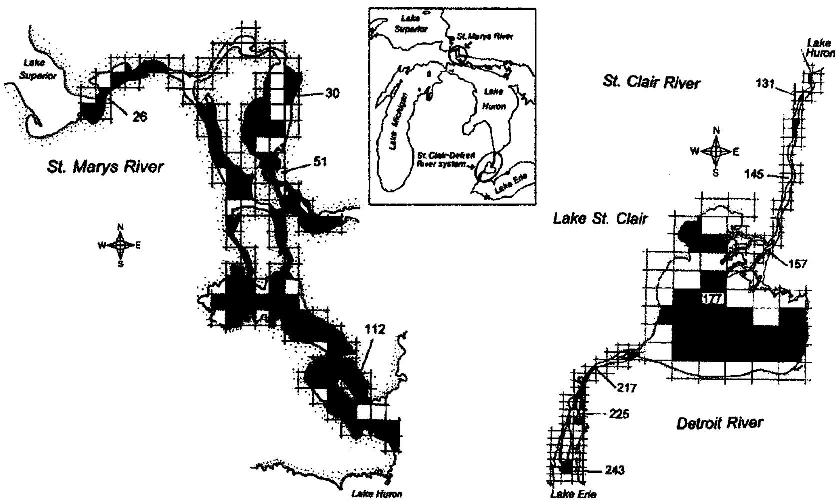

Figure 2. Locations of 250 grid boxes in which 1 station per grid was sampled for *Hexagenia* spp. nymphs in May – June 1985 and locations of 11 sampling stations (26, 30, 31, … 243) where nymphs were collected monthly April through June and August through October 1986 throughout connecting rivers between Lakes Superior, Huron, and Erie. Blackened sections indicate the presence of *Crepidostomum* spp. metacercariae in one or more nymphs in May–June 1985.

Table 1. Percent frequency of occurrence of *Hexagenia* spp. nymphs and *Crepidostomum* spp. metacercariae (cysts within nymphs) in connecting rivers between Lakes Superior, Huron, and Erie of the Laurentian Great Lakes May–June 1985. Number of stations in parentheses. Row values with different superscript numbers are significantly different ($p < 0.05$, χ^2 2 × 2 contingency tests)

	St. Marys River (125)	St. Clair River (35)	Lake St. Clair (43)	Detroit River (47)	Total (250)
Percent of stations with nymphs	88 (110)	80 (28)	72 (31)	75 (34)	81 (203)
Percent of stations with no nymphs	12 (15)	20 (7)	28 (12)	28 (13)	19 (47)
Percent of stations with one or more metacercariae	81 (90)^1	21 (6)^2	68 (21)^1	32 (11)^2	63 (128)
Percent of stations with 1–10 nymphs	25 (27)^1	68 (19)^2	29 (9)	68 (23)^2	38 (78)

metacercariae and a majority of infestation occurred at station 51 in the St. Marys River (Fig. 3, Table 3). No infestation occurred in 96.7% (18069) of the examined nymphs and no infestation was observed in nymphs <6 mm in length, even though 65% of all examined nymphs were <6 mm long (Fig. 3). Of the total 627 infested nymphs, 410 (65%) occurred at station 51 in the St. Marys River (Table 3). Of the total number (14099) of metacercariae 95% (13454) occurred in the 410 infested nymphs collected at station 51 and prevalence, mean abundance, and mean intensity of infestation was higher at station 51 in the St. Marys River than at the other ten stations, except for prevalence at site 30. In addition, station 51 was the only station where a substantial proportion of nymphs (>10%) exhibited ≥25 metacercariae per nymph (Table 4). Therefore, further analyses were performed for nymphs only from station 51 because this was the only site where metacercariae were common enough to show seasonal patterns.

Examination of nymphs from station 51 in the St. Marys River indicated two distinct seasonal periods of infestation that were associated with individual cohorts of the mayfly population (Table 5). Length-frequency distributions of mayfly nymphs indicate the occurrence of three annual cohorts of nymphs (laid as eggs in 1984, 1985, and 1986). The 1984 cohort, deposited as eggs approximately 21 months (July 1984) before April 1986, is difficult to separate from the 1985 cohort

Table 2. Occurrance of *Crepidostomum* spp. metacercariae based on abundance of *Hexagenia* spp. nymphs at individual stations in connecting rivers between Lakes Superior, Huron, and Erie May–June 1985

Number of nymphs/station	Density of nymphs (number/m^2)	Number of stations	Number of stations with metacercariae	Percent of stations with metacercariae
0	0	47	0	0
1	7	16	0	0
2	13	14	1	7
3	21	6	1	17
4	28	5	1	20
5–10	34–69	37	21	57
11–14	75–96	9	8	89
16–19	110–131	5	5	100
20–24	138–165	16	13	81
25–30	172–207	17	14	82
31–40	214–276	17	14	82
41–50	282–344	18	15	83
52–77	358–530	20	16	80
80–100	551–668	11	9	82
116–191	799–1315	11	9	82
450	3099	1	1	100

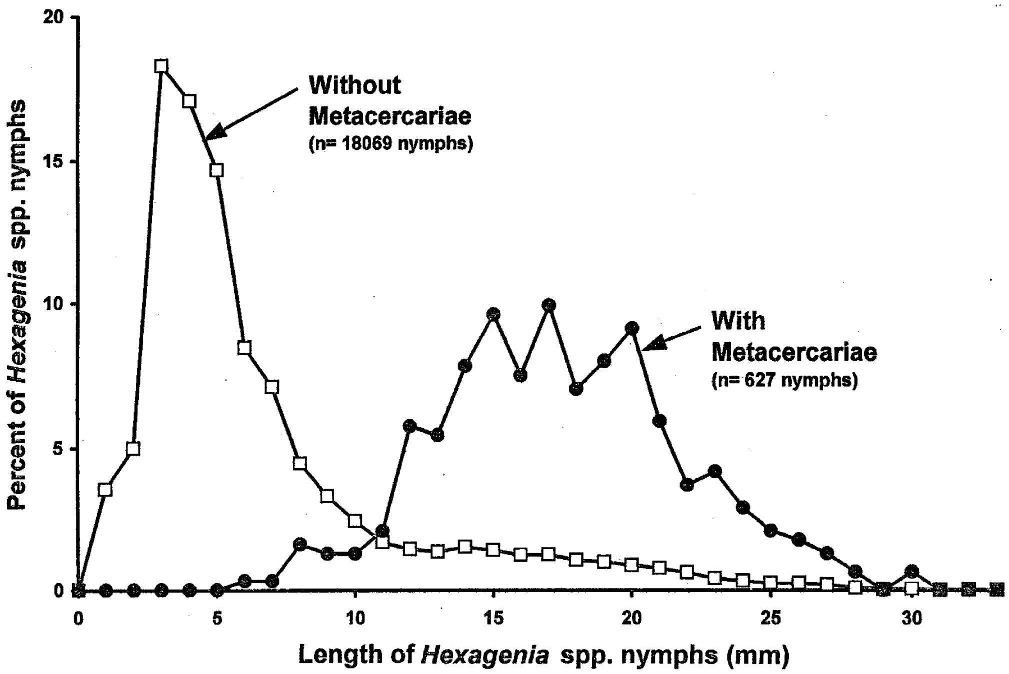

Figure 3. Percent of *Hexagenia* spp. nymphs with and without metacercariae of *Crepidostomum* spp. in whole millimeter length categories of nymphs from 11 stations in connecting rivers between Lakes Superior, Huron, and Erie April through October 1986.

Table 3. Number of infested nymphs, total number of metacercariae, and infestation values of *Crepidostomum* spp. metacercariae in *Hexagenia* spp. nymphs in connecting rivers between Lakes Superior, Huron, and Erie April through October 1986. Total number of nymphs examined in parentheses. Column values with different superscripts are significantly different ($p < 0.05$; prevalence by χ^2 2×2 contingency tests and abundance and intensity by Mann–Whitney U tests)

Station	Number of infested nymphs	Total number metacercariae	Percent prevalence	Mean abundance	Mean intensity
St. Marys River					
26 (1382)	57	102	4.1^{1}	0.1^{1}	1.8^{1}
30 (92)	20	166	21.7^{2}	1.8^{2}	$8.3^{1,2}$
51(1923)	410	13454	21.3^{2}	7.0^{3}	32.8^{3}
112(797)	19	138	2.4^{1}	0.2^{1}	7.3^{2}
Total (4194)	506	13860	12.1	3.2	27.7
St. Clair River					
131(360)	16	32	4.4^{1}	0.1^{1}	2.0
145(812)	0	0	0^{3}	0	0
157(3174)	0	0	0^{3}	0	0
Total (4346)	16	32	0.4	<0.1	2.0
Lake St. Clair					
177(9216)	62	79	0.7^{4}	$<0.1^{1}$	1.3^{2}
Detroit River					
217(327)	12	17	3.7^{1}	0.1^{1}	1.4^{1}
225(219)	30	109	13.7^{5}	0.5^{2}	3.6^{2}
243(394)	1	2	$0.3^{3,4}$	$<0.1^{1}$	2.0
Total (940)	43	128	4.6	<0.1	3.0
Total	627	14099	3.3	0.8	22.5

Table 4. Percent of *Hexagenia* spp. nymphs in six abundance categories of *Crepidostomum* spp. metacercariae at 11 stations in connecting rivers between Lakes Superior, Huron, and Erie April through October 1986

Number of metacercariae	Station								
	26	30	51	112	131	177	217	225	243
1–9	100	80	25	74	100	100	100	93	100
10–24	0	10	23	21	0	0	0	7	0
25–49	0	5	30	5	0	0	0	0	0
50–74	0	5	15	0	0	0	0	0	0
75–100	0	0	4	0	0	0	0	0	0
100–245	0	0	4	0	0	0	0	0	0
Number of infested nymphs	57	20	410	19	16	62	12	30	1

(laid as eggs in July 1985) because of the low number of nymphs collected of the 1984 cohort (43–72 nymphs). In addition, low numbers of infested nymphs (6–13) of the 1984 cohort prevented analysis of infestation values. However, the 1985 cohort and 1986 cohort (laid as eggs in July 1986) are evident in length-frequency distributions and the number of collected and infested nymphs was relatively high.

Infestation of the 1985 cohort was zero in April and low (prevalence <1% and intensity 1.7–1.0 metacercariae/nymph) between April and June (Table 5). Then between June and August, infestation values increased significantly. In addition, a small percentage of nymphs (5%) of the 1986 cohort became infested at low intensities (2.0–4.4 metacercariae/nymph) between August and September.

Infestation was positively related to individual mayfly length (Figs. 3 and 4, Tables 4 and 5). Infestation occurred primarily in larger nymphs (Fig. 3): overall, mean intensity was correlated with nymph length April - October ($r = 0.96$; $p < 0.05$) and significant increases ($p < 0.05$) in mean intensity occurred in five length classes less than 20-mm long (6–11 to 12 mm, 12 to 13, 14 to 15, 16 to 17 and 18 to 19 mm), but no significant decreases were observed (Fig. 4); nymph length and intensity of infestation was significantly correlated for each individual month, except April when few nymphs were collected ($n = 127$ total, 6 infected) (Table 5) ($p < 0.05$, r ranged between 0.60 and 0.77); and, nymph length of the 1985 cohort was positively correlated with prevalence, mean intensity, and mean abundance ($p < 0.05$, $r = 0.67$, 0.98, and 0.97, respectively) and several infestation values increased significantly ($p < 0.05$) with increased nymph length (Table 6).

Discussion

Distribution

The present study is the first to investigate the frequency of occurrence, distribution, and abundance of parasitic metacercariae of *Crepidostomum* spp. in *Hexagenia* spp. mayfly nymphs in a large water body such as the connecting rivers between Lakes Superior, Huron, and Erie, the Laurentian Great Lakes. One study in the Mississippi River looked at the occurrence of metacercariae in winged stages of *Hexagenia* spp. over a large area (ca. 600 km distance) and implied that infestation of nymphs also occurred over the entire area, but no data were presented (Fremling, 1960). A few other studies of mayfly infestation (including *Crepidostomum* spp.) used samples collected at two or three stations in small lakes (Esch & Coggins, 1974; Esch & Hazen, 1982; Moul, 1984; Esch et al., 1986; Marcogliese et al., 1990) and four of those five studies were performed in one small inland lake in Michigan.

The absence of *Crepidostomum* spp. at many stations in several large areas in the present study is attributed to the absence of nymphs and low probability of finding metacercariae at low nymph densities. In general, nymph populations in these areas were low because of pollution (e.g., channel above station 30) and unsuitable substrate (e.g. perimeter of Lake St. Clair) (Schloesser et al., 1991; Schloesser and Hiltunen, 1984). At nymph

Table 5. Length-frequency distributions of three cohorts of *Hexagenia* spp. nymphs (1984 in lower left area, 1985 in middle boxed area, and 1986 in shaded area) and infestion values by *Crepidostomum* spp. in 1985 and 1986 cohorts at station 51 in the St. Marys River April through October 1986. Adjacent infestation values designated by an asterisk are significantly different ($p < 0.05$; prevalence by χ^2 2 × 2 contingency tests and intensity by Mann–Whitney U tests). Correlation coefficients are between nymph length and number of metacercariae

Nymph length (mm)	April	May	June	August	Sept	Oct
1	0	0	0	1	0	0
2	6	3	0	2	18	36
3	23	68	3	12	19	62
4	28	127	23	14	15	7
5	14	170	31	9	39	16
6	9	103	45	5	47	16
7	3	38	48	2	41	14
8	1	21	42	3	19	19
9	1	6	20	1	5	5
10	2	10	17	6	4	10
11	1	6	8	12	9	1
12	2	4	1	11	18	15
13	2	5	6	13	19	14
14	1	5	3	16	24	10
15	2	6	3	12	31	10
16	5	0	1	6	18	12
17	3	3	4	4	28	16
18	2	6	1	5	18	8
19	2	7	5	7	25	13
20	3	3	2	3	23	8
21	4	4	7	2	6	7
22	3	5	3	3	2	5
23	3	4	3	1	3	1
24	2	2	1	0	7	0
25	2	1	5	0	0	1
26	2	0	5	0	0	2
27	1	0	1	0	1	0
28	0	0	2	0	0	0
29	0	0	0	0	0	0
30	0	0	1	0	0	0
31	0	1	0	0	0	0
1985 cohort (boxed area)						
Number nymphs	84	536	238	102	236	123
Number infested nymphs	0	3	1*	46*	211*	93
Percent prevalence	0	<1	<1	45	89	76
Mean Intensity	0	1.7	1.0 *	26.0	33.6	36.8
1986 cohort (shaded area)						
Number nymphs	0	0	0	48	203	185
Number infested nymphs				0	10	9
Percent prevalence				0	5	5
Mean Intensity				0	2.0	4.4
Correlation coefficient	0.69	0.77	0.65	0.60	0.61	0.67

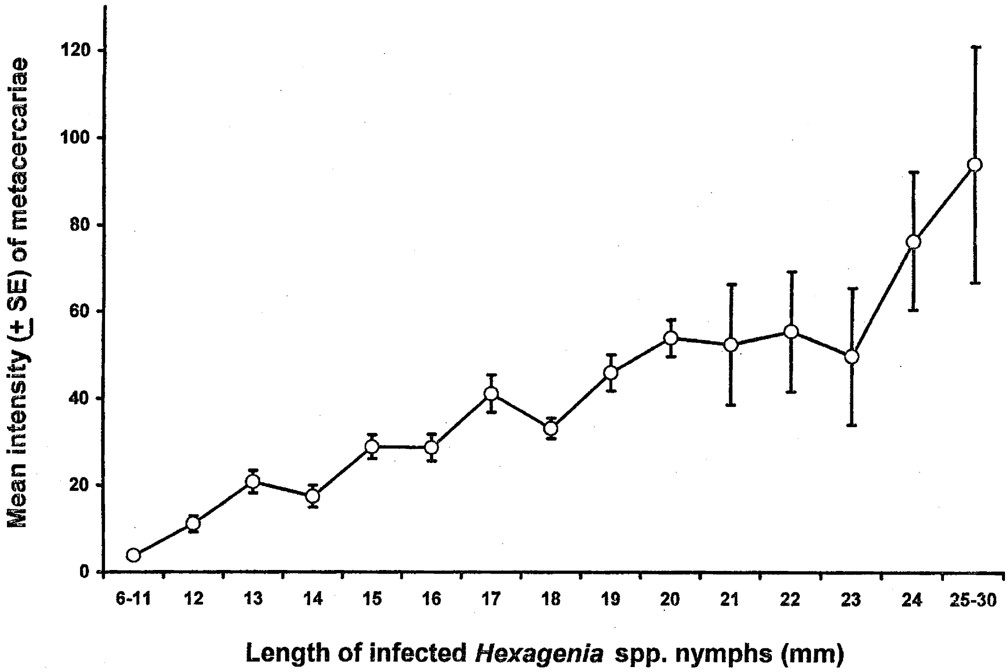

Figure 4. Mean intensity (±SE) of *Crepidostomum* spp. metacercariae in length classes of *Hexagenia* spp. nymphs from station 51 in the St. Marys River April through October 1986. A significant correlation coefficient $r = 0.96$, $p < 0.05$) exists for this relationship and significant increases occurred between 6–11 and 12 mm, 12 and 13, 14 and 15, 16 and 17, and 18 and 19 mm ($p < 0.05$, χ^2 2 × 2 contingency tests).

densities between 7 and 69/m^2 the probability of finding metacercariae was relatively low (mean = 31%, range = 0–57%) which could lead to undetected infestation and distribution. In the St. Marys River, nymphs were absent and in low densities in the channel north of station 30 and in the north half of the lake near station 30 (Schloesser et al., 1991). In these two areas, 80% of the stations ($n = 25$) had nymph densities ≤69/m^2 whereas, low densities only occurred at 22% of

Table 6. Infestation values of *Crepidostomum* spp. metacercariae in the 1985 cohort of *Hexagenia* spp. nymphs at station 51 in the St. Marys River August through October 1986. Infestation values in columns designated with an asterisk are significantly different than the preceding value ($p < 0.05$; prevalence by χ^2 2 × 2 contingency tests and intensity and abundance by Mann–Whitney U tests)

Nymph length (mm)	Number of nymphs examined	Prevalence	Mean intensity	Mean abundance	Variance of abundance	Variance to mean abundance ratio
9	11	55	2.7	1.6	0.92	0.573
10	20	25	7.0	1.2	2.08	1.709
11	22	45	5.8	5.7	8.60	1.513
12	44	75	11.7	8.0*	5.84	0.728
13	46	65	21.4*	14.5	6.61	0.456
14	50	72	18.1	13.4	11.20	0.837
15	53	87	29.73*	24.4*	7.65	0.314
16	36	81	28.6	21.9	18.88	0.863
17	48	88	41.4*	22.2	8.19	0.368
18	31	87	33.9	27.5	17.23	0.627
19	45	72	45.2*	29.0	19.28	0.666
20	34	91	54.5	38.7*	33.40	0.862
21–27	42	76	64.1	42.5	30.90	0.727

stations (n = 100) in the remaining portion of the river (unpublished individual station data summarized in Schloesser et al., (1991)). Similarly, areas that contained large proportions of stations with no (0/m^2) to low densities (7–69/m^2) of nymphs include the ; St. Clair River (71% n = 35 stations), the perimeter of Lake St. Clair (82% n = 22 versus 13% n = 23 at other stations), and the upper and lower Detroit River (91% n = 34 versus 17% n = 13 at other stations). At present, it is not known if additional collection and examination of nymphs from stations at densities ≤69/m^2 would detect additional infestation. However, it is likely examination of more nymphs would reveal more metacercariae, thus increase detection of infestation and distribution. Therefore, it is believed the present study underestimated the distribution of *Crepidostomum* spp. infestation of *Hexagenia* spp. in connecting rivers between Lakes Superior, Huron, and Erie of the Laurentian Great Lakes.

Seasonal abundance

The present study indicates *Hexagenia* spp. nymphs became infested with *Crepidostomum* spp. between June and September. This period of transmission is dependent on the life-cycle of the parasite. In addition, the life-cycle of *Hexagenia* spp. in the St. Marys River determines which cohort of nymphs is infested and therefore, the duration of infestation. A typical life-cycle of *Crepidostomum* (e.g., *C. cooperi*) includes individual stages in fish (definitive host), fingernail clams (first intermediate host), and mayfly nymphs (second intermediate host) (Olsen, 1974; Esch et al., 1986). *Crepidostomum* spp. overwinters between early fall (e.g., September/October) and spring (e.g., May) in first-intermediate hosts and, depending on water temperatures, can be transmitted to second-intermediate hosts (i.e., nymphs) between early and late summer (e.g., June through September) (Esch & Hazen, 1982). Once infested, cysts in nymphs are easily visible within 7 days (Hopkins, 1934; Esch et al., 1986). The life-cycle of *Hexagenia* spp. in the St. Marys River is a two-year cycle (Schloesser & Hiltunen, 1984). Mayfly eggs are laid in July, incubate for 30–45 days, hatch and overwinter as small nymphs (<8–9 mm), rapidly grow the following warm-water season, overwinter a second winter, and emerge as adults in July approximately two years after egg deposition (Hunt, 1953; Schloesser et al., 1984).

Infestation of *Hexagenia* spp. nymphs occurs primarily in the summer after nymphs are recruited into the population (i.e., one year old nymphs). Therefore, most individual nymphs carry parasites for only one of their two year existence. Minimal infestation of young-of-the-year nymphs August through October during the primary transmission period may be due to small body size/exposure, although it is possible different behavior of small and large nymphs could account for different infestation rates of size groups of nymphs. Minimal transmission of parasites to young-of-the-year nymphs is supported by minimal infestation of one year old nymphs of the 1985 cohort in April through June 1986. This cohort would have been exposed to one previous transmission period between nymph recruitment (mid August) and September 1984 when they were young-of-the-year nymphs. Parasite transmission and mayfly emergence that occurs in June and July in the St. Marys River begins during a period of rapidly rising water temperatures. Other researchers have observed similar associations between parasite and host life-cycles, parasite transmission, and water temperature (Awachie, 1968; Hazen & Esch, 1977; Esch & Hazen, 1982).

In general, *Crepidostomum* spp. infestations of nymphs at specific stations in the present study were lower than (10 sites in 1986) or similar to (e.g., station 51) infestation values reported in other studies (Hunt, 1953; Fremling, 1960; Esch & Hazen, 1982; Moul, 1984; Esch et al., 1986; Marcogliese et al., 1990). Although infestation measures have not been consistently analyzed and reported (Bush et al., 1997), a few comparisons to the present study are possible for data collected from Gull Lake, Michigan (Esch & Hazen, 1982; Esch et al., 1986; Marcogliese et al., 1990), Blue Stem Lake, Nebraska (Moul, 1984), and the Sangamon River, Illinois (Hopkins, 1934). Prevalence of metacercariae in nymphs and subimagos in previous studies ranged between 14 and 100% (versus 0.4–4.6% in the St. Clair River, Lake St. Clair and Detroit River and 12.1% in the St. Marys River) and mean abundance ranged between 0.3 and 16.8 per nymph (versus <0.1–0.1 in the three southern water bodies and 3.2 in the St. Marys River) (Moul, 1984; Esch et al., 1986). During comparable periods of time, prevalence of metacercariae in one cohort of nymphs at station 51 (0 to <1% April through June and 45–89% August through October) was similar to those observed in other studies (4–9% spring to early summer and 45–96% mid summer to fall) (Hopkins, 1934; Esch & Hazen, 1982; Moul, 1984). Maximum intensities of metacercariae in individual nymphs from the St. Clair River, Lake St. Clair, the Detroit River, and three stations (26, 30, and 112) in the St. Marys River (<75 metacercariae/nymph) are lower than those reported by Hunt (1953) and Fremling (1960) (185 and 241 per nymph, respectively). However, the maximum intensity of 245 metacercariae in one nymph from station 51 is the highest recorded intensity.

Impacts of parasitism

In the present study, relatively high infestation of *Hexagenia* spp. by *Crepidostomum* spp. at station 51 did not appear to cause mortality of nymphs during the ice-free season of the year. Prevalence and mean intensity and abundance exhibited no pattern of decline with increasing nymph length. Although, parasite induced mortality is difficult to demonstrate in aquatic fauna, declines of these indicators of infestation with increasing length-age of hosts could indicate that larger-older, more infested nymphs die at a faster rate relative to uninfected nymphs (Anderson & Gordon, 1982; Kennedy, 1984; Esch et al., 1986; Marcogliese et al., 1990). However, infestation induced mortality could occur during winter months, especially in the second winter when parasite load is high and mayfly stress may be greatest. Overwinter mortality of the 1984 cohort which had under gone two winter periods and one winter of heavy parasite infestation could not be examined in the present study because of low numbers (6–13) of infested nymphs.

Various studies have suggested that metacercariae may impair body functions and cause non-lethal impacts to mayfly nymphs through decreased proteins, muscle, fat reserves, nerve ganglia, and gonadal tissues which could contribute to retarded growth, declined health, and increase mortality by other factors (Awachie, 1968; Olsen, 1974; Chambers et al., 1975; Knowles & Hall, 1976; Hominick & Welch, 1980; Moravec, 1982). Chambers et al., (1975) found substantial reductions in hemolymph proteins in heavily infected nymphs with metacercarial intensities ranging between 16 and 116 per nymph. Knowles & Hall (1976) noted possible weakening of host defense mechanisms at infestation intensities ≥25 cysts per nymph by another trematode parasite (*Allopodocotyle lepomis*). In the present study, stations 30 and 51 in the St. Marys River were the only stations where infestation intensities were ≥25 cysts (10 and 53% of nymphs, respectively). At station 51, 30% had two-times the suspected impact intensities and 23% of nymphs contained 2–10 times the number of metacercariae likely to cause impaired nymph health. Therefore, it is possible that the health of some nymphs of the *Hexagenia* spp. population in the present study were negatively affected by infestation of *Crepidostomum* spp.

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