

Crepidostomum cooperi (Allocreadidae) in the Burrowing Mayfly, *Hexagenia limbata* (Ephemeroptera) Related to Trophic Status of a Lake

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ABSTRACT.—A long-term study (20 yr) was conducted on aspects of the population biology of *Crepidostomum cooperi* (Trematoda: Allocreadidae) in the burrowing mayfly, *Hexagenia limbata* (Ephemeroptera). The study was in Gull Lake, located in southwestern lower Michigan. During the 1st 16 yr, the lake was subjected to eutrophication. It was hypothesized that the high prevalence (80–98% in most years) and density (range from 4.2–16.8 in females) of metacercariae in subimagos during most of this time were a consequence of eutrophication. The process forced mayfly nymphs away from hypolimnetic substrata, during periods of thermal stratification and anoxia, into shallow areas of the lake where transmission dynamics of the parasite would be heightened between the first (sphaeriid clams) and second (mayfly nymphs) intermediate hosts. Reversal of eutrophication was expected with completion in 1984 of a sewer system for the Gull Lake drainage basin. Substantive changes in parasite population dynamics were predicted to occur with reversal of eutrophication. In 1984, prevalence of metacercariae in female subimagos was 99.0% and 90.6% in males; by 1989, prevalence in females had declined to 35.8% and to 25.0% in males. Mean density in females in 1984 was 16.5 and 5.6 in males. In 1989, mean density was 1.1 in females and 0.3 in males. These observations confirm the earlier prediction; we suggest that transmission dynamics of the parasite have been influenced primarily by density-independent factors which have affected the spatial distribution of nymphs in the lake's substrata.

INTRODUCTION

Esch *et al.* (1986) reported the results of a 16-yr study on various aspects of the population biology of the allocreadid trematode, *Crepidostomum cooperi*, in Gull Lake, located in southwestern lower Michigan. The parasite matures in centrarchid fishes, uses sphaeriid clams as first intermediate hosts, and *Hyalella azteca* (Amphipoda) and *Hexagenia limbata* (Ephemeroptera) as second intermediate hosts. Esch *et al.* emphasized that long-term investigations such as this are rare and, as such, their results should be considered cautiously for two reasons. First, there are few data bases with which the results of their study could be compared. Second, and perhaps more importantly, the nature of the study made replication and controls impossible. Accordingly, the relationships postulated were necessarily based on inferences generated from the long-term parasite data set in conjunction with predictions regarding dispersal and colonization patterns of mayfly nymphs in the lake during eutrophication and its reversal.

As a consequence of the eutrophication, Esch *et al.* (1986) noted that all of the hypolimnetic substrata in the lake become anoxic during the period of normal thermal stratification and, as a result, were no longer suitable for colonization by mayfly nymphs. They speculated that nymphs had been forced to occupy shallower areas of the lake which, in turn, had brought them into greater physical overlap with the first intermediate hosts (sphaeriid clams)

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of the parasite. Because of this habitat shift, they suggested that the probability of parasite recruitment by the nymphs had been enhanced, increasing both the prevalence and intensity of infection by the parasite in nymphs during the period of eutrophication. In support of this argument, they pointed out that the mean densities of metacercariae in male subimagoes had increased from 1.6 in 1969 to 5.6 in 1984; in females, densities increased from 4.2 to 16.5 over the same time period. They predicted that with reversal of the eutrophication process, the population dynamics of the parasite in the mayflies would be altered and, ultimately, both prevalence and density of metacercariae in *Hexagenia limbata* subimagoes would decrease.

In order to reverse eutrophication, a reduction in phosphate loading was necessary. In 1984, construction of a sewer system for the Gull Lake drainage basin was undertaken to effect such a change. Esch *et al.* (1986) predicted that changes in water quality would require 5–10 yr in order to alter the dispersal pattern of the mayfly nymphs in the lake's substrata and, therefore, the population biology of the parasite in the mayfly. Based on analysis of data generated from 1986 through 1989, we now suggest that the 5–10 yr component in their predictions was in error and that, more importantly, the prediction regarding the dynamic aspect of the parasite's transmission process was correct.

MATERIALS AND METHODS

Subimagoes of *Hexagenia limbata* were collected after emergence in August of each year from 1986 through 1989, as had been done in August during each of the previous 16 yr. Nymphs were also collected from the sediments at two sites in early July 1987, as they had been in July 1984 (Esch *et al.*, 1986). One of the collecting sites was at the NW corner of Gull Lake (NP) and the other was at Ross Township Park (RTP), adjacent to the W. K. Kellogg Biological Station. Thirty grabs with a 15.2 cm Ekman dredge were made at depths of 3, 5 and 7 m at each site. Grab samples were washed through a series of hardware cloth screens with the smallest mesh size of 1.5 × 2.0 mm. All nymphs and subimagoes were placed into 70% ethanol and returned to the laboratory where metacercariae were counted and hosts sexed.

The water quality characteristics of Gull Lake have been described by Moss (1972), Tague (1977) and Esch *et al.* (1986). In brief, the lake underwent eutrophication prior to 1984 when a sewer system was constructed to reduce the input of excessive phosphates, primarily from faulty septic systems in the lake basin. A recent communication from Dr. George Lauff, Director, W. K. Kellogg Biological Station, indicates that the phytoplankton community in the lake has changed from that reported by Moss (1972) and Tague (1977) and that the eutrophication process appears to have been stopped, if not reversed.

Mean density of metacercariae is defined as the mean number of metacercariae per mayfly, including both infected and uninfected individuals. Prevalence is the percentage of infected mayflies. Based on known size differences between male and female subimagoes, and the previously demonstrated (Esch and Hazen, 1982) correlation between host size and parasite density, analyses of both prevalence and density patterns were undertaken separately for each sex. Except where specifically noted, densities are expressed as $\bar{x} \pm S^2$.

RESULTS

The prevalence of *Crepidostomum cooperi* in male subimagoes was consistently over 80% between 1969 and 1984 (*see also*, Esch *et al.*, 1986), with the years 1969, 1972, 1980 and 1982 being exceptions to the long-term trend (Fig. 1). Among female subimagoes during the same time frame, prevalence was consistently over 90%; exceptions to this pattern occurred in 1969, 1972 and 1980. However, prevalence of the parasite in subimagoes of

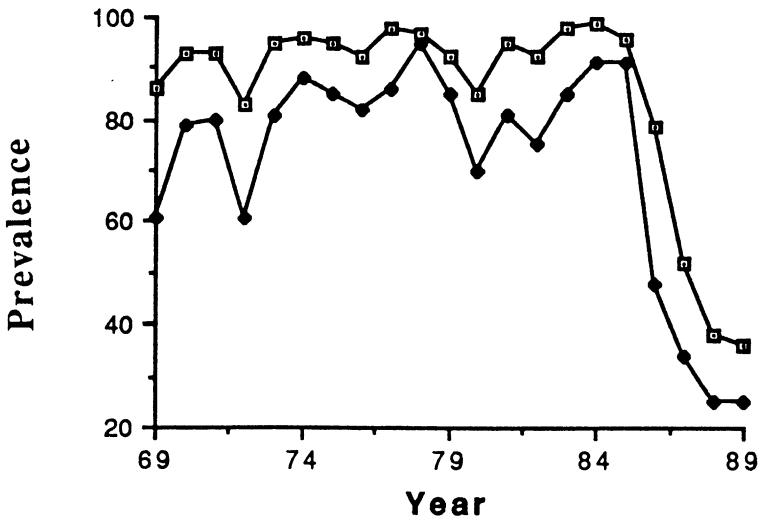


FIG. 1.—Prevalence of *C. cooperi* metacercariae in male (closed squares) and female (open squares) subimagos from 1969 through 1989 in Gull Lake

both sexes declined sharply from 1986, reaching 25.0% in males in 1988 and 37.6% in females during the same year; in 1989, prevalence was 25.0% in males and 35.8% in females.

Density of metacercariae in female subimagos over the 1st 16 yr exhibited two peaks, the first in 1976 and again in 1984 (Fig. 2). The mean density of metacercariae in female subimagos was <5.0 only once between 1969 and 1984, and that was in the 1st yr of the study. By 1988, however, the mean density had declined to 1.03 parasites/female host, the lowest level over the entire 20 yr. The pattern of change among male subimagos was virtually identical to that for females, with a low density of 0.4 occurring in 1988 (Fig. 2); in 1989, the mean density in males was 0.3 and 1.1 in females.

The prevalence and density of *Crepidostomum cooperi* metacercariae in male and female nymphs were measured from the same two sites as in 1984 (Esch *et al.*, 1986) in early July 1987. Highest prevalences in male nymphs at NP and RTP were at 5 m (Table 1). Highest mean densities among male nymphs were at 7 m from NP and at 5 m from RTP. Among female nymphs (Table 1), the highest prevalences for both NP and RTP were at 5 m. Highest mean densities at NP in female nymphs were at 5 m and at 7 m from RTP. There was no obvious pattern of prevalence or density with change in depth among female or male nymphs except at NP where mean density in males increased with increasing depth and at RTP where density in females increased with increasing depth.

Metacercariae were contagiously distributed among nymphs of both sexes in July 1984 (Fig. 3; *see also*, variance/mean ratios in Esch *et al.*, 1986). However, the mean densities and the degree of contagion among subimagos of both sexes collected in August 1984, were clearly much greater, indicating that parasite recruitment continued from early July into August until the time of subimago emergence from the lake.

In July 1987, the frequency distribution of metacercariae among male and female nymphs (Fig. 4) was virtually identical to that observed for metacercariae in both male and female nymphs in July 1984 (Fig. 3). In sharp contrast to 1984, however, the frequency distribution pattern of metacercariae among male and female subimagos collected 6 wk later in 1987 was essentially unchanged from that seen among nymphs collected in July. Moreover, the

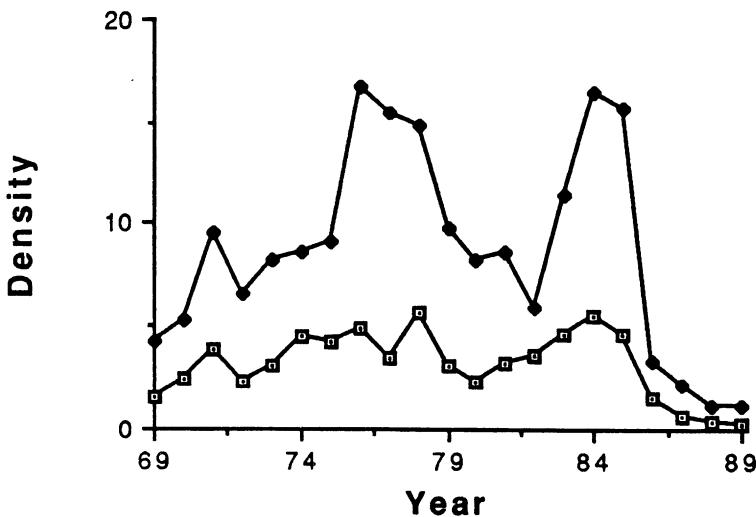


FIG. 2.—Density of *C. cooperi* metacercariae in female (closed squares) and male (open squares) subimagoes from 1969 through 1989 in Gull Lake

mean density of metacercariae in subimagoes collected in August was actually less ($\bar{x} = 2.27 \pm 17.98$) than the abundance of metacercariae in nymphs (3.27 ± 128.87) collected a month earlier. These observations tend to suggest that parasite recruitment which occurred was negligible during this period in 1987.

DISCUSSION

The long-term increases and then the sharp decline in both prevalence and density of metacercariae reported herein and by Esch *et al.* (1986) could have been influenced by density-dependent population regulation (*sensu* Anderson, 1978; Anderson and May, 1978; Scott and Dobson, 1989). However, Esch and Hazen (1982) examined the possibility of density-dependent, parasite-induced host mortality and rejected the notion using data obtained from metacercariae distribution patterns in both *Hexagenia limbata* and the amphipod, *Hyalella azteca*. According to Anderson (1978), several processes are important in producing a "stable dynamical equilibrium," or regulation. Of these, only one, a contagious frequency distribution of metacercariae among subimagoes, could be associated with the *Crepidostomum cooperi*/*Hexagenia limbata* system. This is certainly not sufficient grounds for concluding that density-dependent population regulation is affecting *H. limbata* via a negative impact by *C. cooperi* metacercariae. Moreover, it is doubtful that most parasites act as regulators of host population dynamics except very rarely (Lemly and Esch, 1984). Even in the few host/parasite systems in which mortality has been observed, death was usually associated with alterations in host behavior (*i.e.*, hosts became more vulnerable to predation following parasite infection) rather than to the direct negative effects of a parasite on its host (Dence, 1958; Arme and Owen, 1968; Pennycuik, 1971; Wilson, 1971; Brassard *et al.*, 1982).

Alternatively, Esch *et al.* (1986) proposed that changes in physicochemical characteristics in the lake, brought on by eutrophication, were the primary factors in effecting the observed changes. These sorts of density-independent factors are similar to those inferred by Kennedy and Rumpus (1977) as being responsible for maintaining consistency of the acanthocephalan,

TABLE 1.—Prevalence and mean densities of *C. cooperi* in mayfly nymphs from RTP and NP

Location (depth)	Sex*	Prevalence	$\bar{x} \pm S^2$ parasite density
RTP(3)	Male (41)	25.6%	0.41 \pm 0.72
RTP (3)	Female (7)	14.3%	0.29 \pm 0.49
RTP (5)	Male (41)	70.8%	1.73 \pm 3.42
RTP (5)	Female (26)	73.3%	2.62 \pm 7.67
RTP (7)	Male (26)	48.0%	1.27 \pm 2.72
RTP (7)	Female (104)	53.7%	3.56 \pm 178.76
NP (3)	Male (5)	80.0%	1.00 \pm 1.21
NP (3)	Female (9)	77.8%	4.33 \pm 22.18
NP (5)	Male (23)	83.9%	2.78 \pm 10.96
NP (5)	Female (18)	82.6%	4.78 \pm 32.15
NP (7)	Male (15)	73.3%	5.87 \pm 43.69
NP (7)	Female (11)	72.7%	3.45 \pm 8.41

* Number sampled

Pomphorhynchus laevis, in the amphipod, *Gammarus pulex*, and in dace, *Leuciscus leuciscus*, from the River Avon in England. In a study lasting 20 yr in Babine Lake in Canada, Smith (1973) also observed remarkable consistency in the prevalence of the cestode, *Eubothrium salvelini*, in smolts of *Onchorhynchus nerka*. The two periods when significant deviations from the norm in prevalence occurred were attributed to the asynchronous appearance of intermediate and definitive hosts in the lake, again, a density-independent phenomenon.

Inconsistency in prevalence and density of metacercariae of two digenetic trematodes infecting mosquitofish *Gambusia affinis* in a South Carolina cooling reservoir (Aho *et al.*, 1982; Camp *et al.*, 1982) was attributed to irregular temperature fluctuations in the reservoir, or to variability in the nesting or foraging behavior of definitive hosts, either of which would be considered as density-independent regulatory phenomena. Granath and Esch (1983), Riggs and Esch (1987), and Marcogliese and Esch (1989) conducted a series of studies lasting 7 yr on several aspects of the population biology of the Asian tapeworm *Bothriocephalus acheilognathi* in a North Carolina cooling reservoir. The reservoir had been subjected to severe selenium pollution, leaving the main body of the lake devoid of piscine predators. In this reservoir, long-term changes in the population biology of the tapeworm were attributed to alterations in the zooplankton community structure which were brought about by introduction of the planktivorous red shiner *Notropis lutrensis* into an already depauperate fish community (Marcogliese and Esch, 1989). Again, this would be an example of long-term mediation via density-independent factors. In most of these cases, alterations in parasite prevalence and density were affected by modifications in transmission dynamics within the system; prevalence and density were, therefore, primarily influenced by the spatial-temporal dimensions of the parasite's transmission "window" (Kennedy, 1987; Esch *et al.*, 1988).

We suggest that changes in the spatial dimensions of the transmission "window" have had a profound impact on the long-term population dynamics of *Crepidostomum cooperi* in Gull Lake mayflies. Esch *et al.* (1986) proposed that the colonization or dispersal pattern of mayfly nymphs gradually increased the likelihood of parasite recruitment during the period of eutrophication. Because of the reversal of eutrophication since 1984, we propose that the distribution of *Hexagenia limbata* nymphs has been altered to such an extent that the probability of parasite recruitment by these potential hosts has been significantly reduced. The decline in parasite prevalence and density is probably due to the increased availability of colonization sites in the substrata covered by hypolimnetic water which reduced overlap

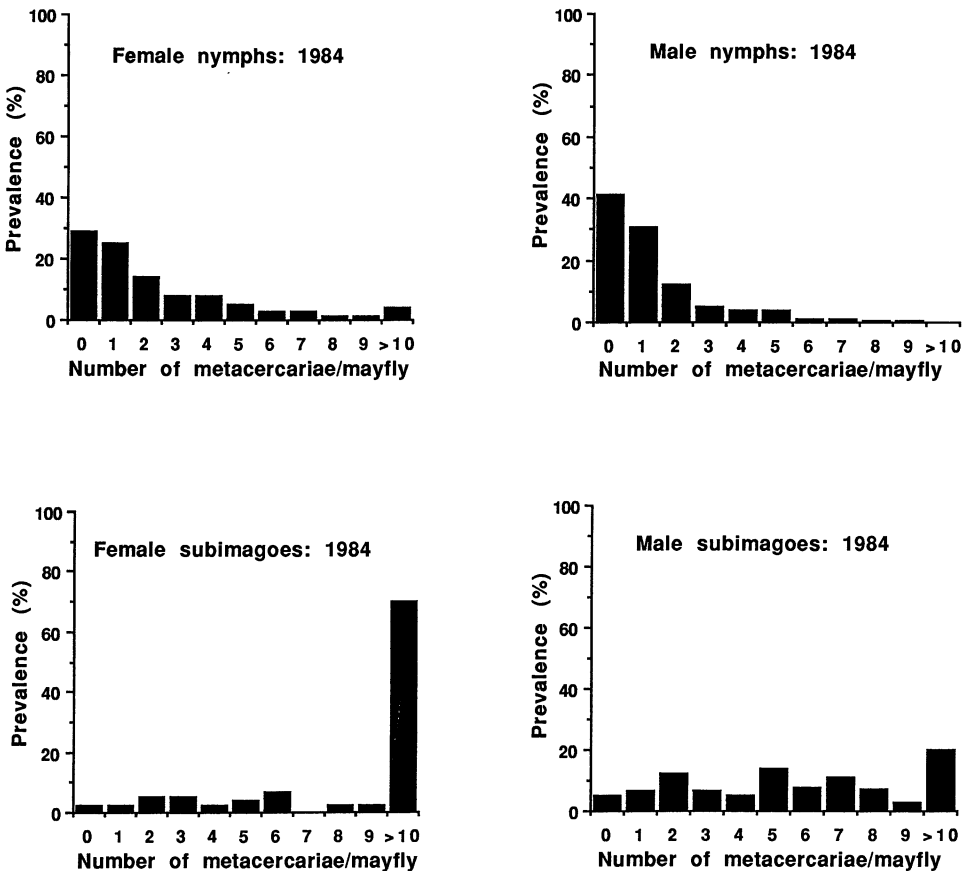


FIG. 3.—Frequency distributions of *C. cooperi* metacercariae among male and female nymphs collected in early July 1984 vs. frequency distributions of *C. cooperi* metacercariae among male and female subimagoes collected in August 1984

between mayfly and sphaeriid clam populations. In 1984, the substrata at each of six hypolimnetic sites were foul-smelling, an indication of anaerobic conditions deep in the water column of the hypolimnion. While we cannot be certain of the presence or absence of oxygen at these sites in July 1987 because of malfunctioning sampling equipment, the substrata were no longer foul-smelling, a clear indication of change in the system.

Nymphs collected from NP and RTP in 1984 (Esch *et al.*, 1986) exhibited consistent patterns of metacercariae prevalence and density with nymphs from shallower sites having higher prevalence and densities of the parasite. Those observations were used to support the hypothesis regarding parasite transmission dynamics and intermediate host dispersal in shallow vs. deep water during the period of eutrophication. We had hoped to show that by sampling at the same two sites following construction of the sewer system, parasite prevalence and densities would have been reversed from the earlier sampling period, but the data are inconsistent with this notion. Thus, there are clear inconsistencies in the depth-prevalence-density patterns at both sites as compared to 1984. We have no explanation for the 1987

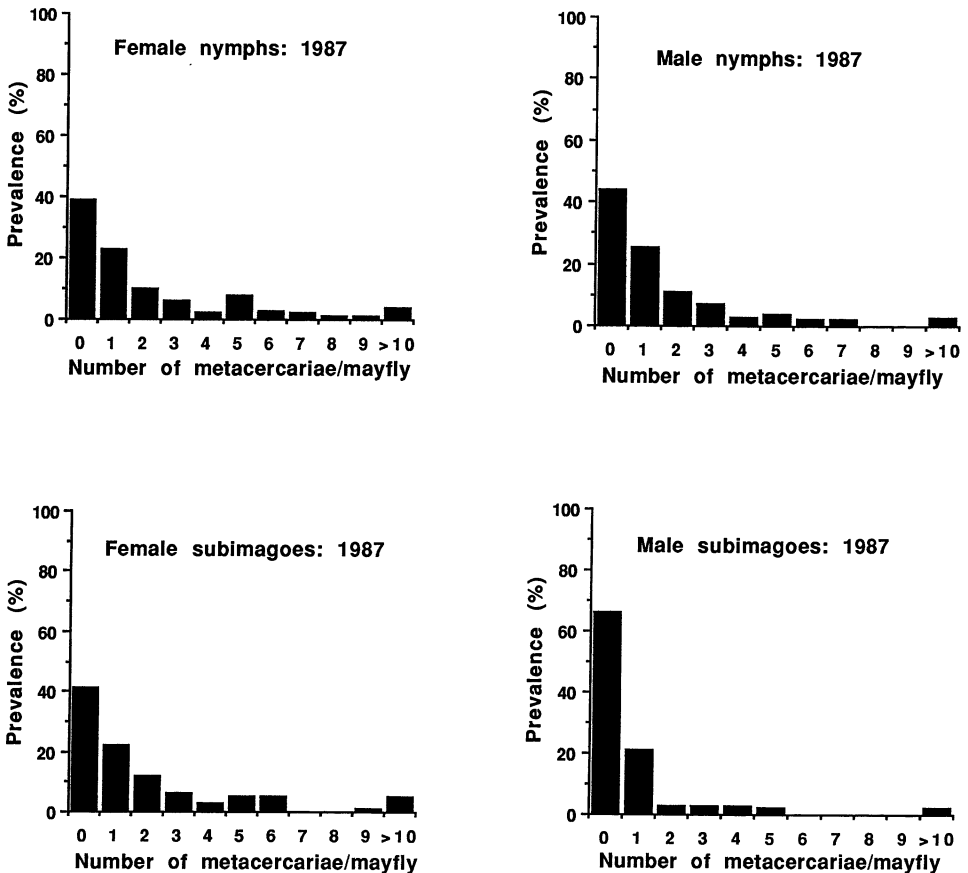


FIG. 4.—Frequency distributions of *C. cooperi* metacercariae among male and female nymphs collected in early 1987 vs. frequency distributions of *C. cooperi* metacercariae among male and female subimagos collected in August 1987

observations except to note that the sample sizes at three of the sampling stations were <10 and this could have biased the results. On the other hand, it is conceivable that the reversal of the eutrophication process is affecting the benthic community structure in a manner similar to the phytoplankton (Dr. George Lauff, pers. comm.) and, probably, the zooplankton community structure. If this is the case, then we would expect to see alterations in the distribution pattern of nymphs in both the hypolimnetic and epilimnetic substrata from 1984 to 1987.

Subimagos emerge from Gull Lake during the evening and night-time hours after about 1 August of each year. At the time of emergence and before the final molt to become sexually mature adults, they are attracted to light and can be easily collected from window screens, at the bases of light poles, etc. These subimagos would represent a mix from many areas of the lake, both deep and shallow; they are not representative of a single lake site or a single depth such as were the July sample of nymphs from either RTP or NP. It is thus of interest to note that the mean density of metacercariae in female nymphs from July 1987

($\bar{x} = 3.27$) is actually higher than in female subimagoes collected 6 wk later ($\bar{x} = 2.27$). This is an important observation since we also know that cercariae are shed from sphaeriid clams continuously from July into August, and even beyond (Hazen and Esch, 1977; Esch and Hazen, 1982). We interpret the lack of difference in densities of metacercariae in the July and August (1987) collections to indicate that the subimagoes collected in August included substantial numbers from areas of the lake where parasite recruitment was proceeding at a very low rate, e.g., the deeper areas of the lake where transmission rates were lower because of reduced numbers of sphaeriid clams. The method of collecting subimagoes has been consistent in each of the 20 yr of the study. This means that the August 1984 collection of subimagoes prior to the reversal of eutrophication also represented a mixed sample. If our idea regarding the differential distribution of mayfly nymphs before and after eutrophication reversal is correct, then the subimagoes collected in 1984 and in all years during the period of eutrophication were apparently from shallower parts of the lake where transmission was taking place at a much higher rate.

The notion that parasite transmission into the mayfly population in 1987 was reduced is confirmed by comparing the frequency distribution data in 1987 and 1984. In July of both years, metacercariae frequency distributions in nymphs were virtually identical. In August 1984, parasites exhibited a much greater degree of contagion in subimagoes as compared with nymphs from 6 wk earlier (Esch *et al.*, 1986). In August 1987, on the other hand, the degree of contagion in subimagoes was not different from that observed in nymphs 6 wk earlier, indicating that far less parasite recruitment into the mayfly population had occurred during that period of time.

We contend that the reversal of the eutrophication process in Gull Lake has significantly modified the opportunities for site selection by the mayfly nymphs and, consequently, has altered the nature of *Hexagenia limbata*-*Crepidostomum cooperi* interactions. Moreover, we conclude that this change has occurred much more rapidly than originally predicted (Esch *et al.*, 1986). We would now predict that these changes in prevalence and density will move toward some, as yet unknown, equilibrium point while the lake continues to return to its more pristine oligotrophic state. Indeed, it is possible that the equilibrium point has already been reached since prevalence and density were virtually identical in subimagoes of each sex in both 1988 and 1989. We do not predict that the parasite is in danger of local extinction since it also occurs in relatively high numbers in the amphipod, *Hyaella azteca* (Hazen and Esch, 1977).

Acknowledgments.—Publication of this research is supported by a grant from the Wake Forest University Research and Publication Fund. We gratefully acknowledge the assistance of Dr. George Lauff and Mr. Art Weist during the entire 20 years of the study.

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