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SEASONAL PATTERNS IN THE BIOLOGY OF EUBOTHRIUM
SALVELINI INFECTING BROOK TROUT AND SLIMY SCULPIN
FROM SWEETWATER CREEK, MICHIGAN

presented by

Alexander D. Hernandez

has been accepted towards fulfillment
of the requirements for

M.S. degree in Zoology

Patrick M. Muzzall
Major professor

Date 8/6/97

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SEASONAL PATTERNS IN THE BIOLOGY OF EUBOTHRIUM SALVELINI
INFECTING BROOK TROUT AND SLIMY SCULPIN FROM
SWEETWATER CREEK, MICHIGAN

By

Alexander D. Hernandez

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Zoology
&
Interdepartmental Program in Ecology, Evolution and Behavioral Biology

1997

ABSTRACT

SEASONAL PATTERNS IN THE BIOLOGY OF EUBOTHRIUM SALVELINI INFECTING BROOK TROUT AND SLIMY SCULPIN FROM SWEETWATER CREEK, MICHIGAN

By

Alexander D. Hernandez

A total of 392 brook trout, Salvelinus fontinalis, and 211 slimy sculpin, Cottus cognatus, was examined for the intestinal cestode Eubothrium salvelini from May 1995 through September 1996 from Sweetwater Creek, Michigan. The distribution of E. salvelini is aggregated in brook trout and may be due to differences in parasite prevalence in fish of different length but not sex. There is a positive correlation between host length and number of worms in large piscivorous fish. In slimy sculpin the parasite distribution is also aggregated but is not due to differences in parasite prevalence or intensity in fish of different length or sex. No seasonal pattern in prevalences and intensities of parasites in brook trout and slimy sculpin was determined. A seasonal pattern of changes in the size and structure of E. salvelini was examined in terms of input, output and control factors. A seasonal pattern in maturation is present in E. salvelini in brook trout which suggests that recruitment (input) of this parasite occurs during late Summer and early Fall. During this time the infective stage would be expected to be available in the environment for fish to recruit new worms but a total of 6399 copepods, the intermediate hosts, were not infected. No maturation of worms occurs in slimy sculpin which suggests that this is a paratenic or dead end host. Output factors could not be measured. Control factors do not appear to be important in this system. Stomach analyses show that large brook trout feed more often on sculpin than they do on copepods which suggests that the aggregated distribution of E. salvelini in trout may be due to large fish feeding on infected slimy sculpin. Therefore, slimy sculpin may play an important role in the life cycle of E. salvelini as a paratenic host.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my major professor, Dr. Patrick M. Muzzall, for giving me an opportunity to come to Michigan State and work under his tutelage. Also, his advice, support, patience and constructive criticisms throughout this study are greatly appreciated. My guidance committee, Drs. Donald J. Hall and Thomas G. Coon, provided me with their support and useful suggestions which were critical in completing this study.

I would like to acknowledge Tom Rozich, field biologist at the Michigan Department of Natural Resources in Cadillac, Michigan, for permission to collect fish from Sweetwater Creek. Funds for this study were provided by the Department of Zoology and the Interdepartmental Program in Ecology, Evolution and Behavioral Biology.

This study could not have been completed without the assistance of the following people: Tom Coon and Tammy Newcomb for permitting me to use their electroshock unit early in this study. Valerie Brady and Brad Cardinale in helping me figure out an effective way to collect copepods. Audrey Armoudlian, Chris Aurbach, Puja Batra, Valerie Brady, Brad Cardinale, Beth Dankert, Randy DeJong, Anne Engh, Tammy Newcomb and Tim Work for assisting me in the collection of fish.

I am grateful to friends and fellow graduate students for participating in discussions of my work. Your company and moral support made life in East Lansing very enjoyable during these past years. Randy DeJong provided me with useful comments and valuable support during our numerous and lengthy discussions about work, ecology and life in general. Thanks for listening. Micaela Szykman was an incredible source of support

during my years here at Michigan State. Our time together was like a roller coaster, full of ups and downs, but in the end I could not have found a better friend than her.

Lastly, I would like to thank my mother for her patience, love and endless support of my work. Everything I am, have been and ever will be I owe to her.

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INTRODUCTION

Two hypotheses have been postulated to explain the determinants of species richness among intestinal parasitic helminths and are reviewed by Bush et al. (1990). The first, 'host age' or phylogenetic hypothesis, predicts that evolutionarily older hosts will have more parasite species than an evolutionarily younger host. Those host species that have been around for a longer period of time have had more time to accumulate a greater variety of helminths species. However, patterns of species richness among intestinal helminths show that the mean number of species is greater in aquatic birds and mammals (the younger taxa) than fish (the older taxa) (Bush et al., 1990).

The second hypothesis, 'host capture' as a determinant of species richness, predicts that evolutionarily younger hosts will have a greater helminth species richness as a result of "capturing" them from older or contemporary hosts. There exists examples of mammals and birds having captured certain helminths from fish (Hoberg, 1987), birds from mammals (Chabaud, 1965), mammals from birds (Barlett and Greiner, 1986) and even reptiles from fish (Bush et al., 1990). When taxon is ignored, aquatic vertebrates harbor a greater mean number of intestinal helminth species than terrestrial vertebrates (Bush et al., 1990). The results of these studies suggest that the capturing of these helminths is primarily determined by ecology (biotic and abiotic factors) (Esch, 1971) and not evolutionary time. Specifically, factors such as trophic interactions between various animals that serve as intermediate and definitive hosts, distribution of hosts, and season of host collection may all influence the helminth species composition (i.e. species richness) in a particular ecosystem (Dogiel, 1962; Esch, 1971).

Species richness refers to the number of parasite species infecting a particular population of hosts and can be one measurement used to determine the diversity of the parasite community. Parasite communities are hierarchical in nature (Holmes and Price, 1986; Esch et al., 1990) and this hierarchy is composed of several levels. All parasite species, in all of their host species, from an ecosystem are termed the compound community (Root, 1973; Holmes and Price, 1986). This compound community is in turn made up of the component community which is defined as all of the parasites in a given host species' population (Root, 1973; Holmes and Price, 1986). This level of organization can be further broken down to the infracommunity which is made up of all parasites in one host (Holmes and Price, 1986). Lastly, the infracommunity can be broken down to the infrapopulation which is made up of all individuals of a species of parasite occurring in one host (Margolis et al., 1982).

It is at the infrapopulation level that the proximate mechanisms that may determine patterns of species richness in an ecosystem can begin to be measured. However, the impracticality of studying changes in the size and structure of parasite populations in individual fish makes the study of these populations from natural systems difficult. Therefore the definition of the parasite population unit requires the formulation of an important assumption. Kennedy (1970) proposed that patterns in the changes in the size and structure of parasite populations may be revealed by taking regular samples from the host population, assuming that any patterns are typical of the parasite population and that they are similar in all the hosts.

The distribution of parasites within the host population is often overdispersed and aggregated (Crofton, 1971) (i.e. few of the hosts harbor most of the parasites). This pattern is seen in fish populations (Kennedy, 1968; Kennedy and Hine, 1969) and can sometimes be explained by the influences of fish sex and size (Dogiel et al., 1961; Kennedy, 1970). Finding differences in the parasite population due to fish sex or size may indicate that the size and structure of the parasite population in all fish may not be similar.

General systems theory was first introduced to the field of experimental biology by Quastler (1965) and later to parasitology by Ratcliffe et al. (1969). It was implemented by Kennedy (1970) to study changes in the flow of intestinal parasites through fish as the definitive hosts and the factors that influence this process. In his review of several British freshwater fish, no single pattern was found to exist. This systems approach suggests that changes in size and structure of the parasite population will be influenced by a variety of input, output and control factors and that these factors can vary in time (Kennedy, 1970). Input factors may be the availability of larvae in the environment, variation in the diet and feeding habits of the fish. Output factors may be the parasites failure to establish themselves, rejection of the larvae by the host, and parasite senescence. Control factors may be biotic such as inter and/or intraspecific competition or they may be abiotic such as variation in water temperature. Temporal changes in these factors and/or their interaction may reveal a seasonal pattern in the size and structure of parasite populations which may reveal seasonal differences in the structure of the parasite community and in parasite richness.

The cestode Eubothrium salvelini (Shrank, 1790) (order Pseudophyllidea, family Amphicotyliidae) is holartic and circumpolar in its distribution and is common in salmonid fish. It has a heteroxenous (indirect) life cycle with copepod intermediate hosts (Vik, 1963; Boyce, 1974; Kennedy, 1978a; Poulin et al., 1992) and fish definitive hosts. Adults live in the fish caeca and small intestine. Embryonated eggs are passed with the feces and can survive in the water for at least 30 days at a temperature of 5 °C (Boyce, 1974). The eggs are ingested by copepods where the oncospheres hatch and penetrate the gut and move into the haemocoel. Within 25 to 30 days post egg ingestion, the oncospheres develop into infective proceroids. Fish become infected by ingesting infected copepods and once in the fish, development of the worm to the adult stage occurs. Segmentation does not occur until 30 days later, adults reaching reproductive maturity after 5 months.

Copepods of the genus Cyclops have been reported as intermediate hosts for E. salvelini (see Vik, 1963). Smith (1973) reported C. scutifer as the intermediate host in lakes. Cyclops scutifer, C. bicuspidatus and C. vernalis have been experimentally infected with E. salvelini but only C. scutifer were found naturally infected (Boyce, 1974).

Two races of E. salvelini were described by Kennedy (1978a): the European race as specific to the arctic charr (Salvelinus alpinus) and the American race as less host specific and infecting Salvelinus, Salmo, Oncorhynchus, Mylocheilus, Ptychocheilus. The occurrence of this parasite in North American fish has been summarized by Hoffmann (1967) and Margolis and Arthur (1979). It was first reported in Lake Michigan in brown trout (Salmo trutta), lake trout (Salvelinus namaycush), bloater (Coregonus hoyi) and rainbow trout (Oncorhynchus mykiss) (see Amin, 1977), and recently from alewives (Alosa pseudoharengus) (see Muzzall, 1994). The first record in brook trout (Salvelinus fontinalis) in North America was by Cooper (1918) and its distribution in this species in North America and Michigan was summarized by Muzzall (1984, 1993a).

Eubothrium salvelini can have a detrimental effect on fish. This parasite can lead to altered blood composition of the fish (Hoffmann et al., 1986), reduced swimming performance (Boyce, 1979), along with reduction in resistance to environmental stress (Boyce and Yamada, 1979; Boyce and Clarke, 1983), condition, growth and survival (Smith, 1973; Boyce, 1979; Bristow and Berland, 1991). It affects copepods (C. vernalis) by altering their swimming behavior, possibly making them more susceptible to predation by brook trout (Poulin et al., 1992).

Recently E. salvelini was reported from wild populations of brook trout and slimy sculpin (Cottus cognatus) in Sweetwater Creek, a small first order creek in west central lower Michigan that flows into the Pere Marquette River which empties into Lake Michigan (Muzzall, 1993a). Eubothrium salvelini were found in resident brook trout and slimy sculpin but not in resident populations of brown trout or young chinook salmon (O. tshawytscha), coho salmon (O. kisutch) and rainbow trout from the Pere Marquette River.

Adult chinook salmon, coho salmon and rainbow trout infected in Lake Michigan with E. salvelini migrate into the Pere Marquette River to spawn but do not move into and spawn in this area of Sweetwater Creek. It was suggested that the appropriate intermediate host(s) of E. salvelini are present in Lake Michigan and Sweetwater Creek but absent in the Pere Marquette River (Muzzall, 1993a). An important observation from this study is that brook trout and slimy sculpin residing in this section of Sweetwater Creek were hosts to only one intestinal parasite, E. salvelini.

Finding an aquatic ecosystem in which there exists a one intestinal parasite species-one host species interaction, especially one involving a salmonid, is very infrequent. To my knowledge there are no such published reports from the North American fish parasite literature. European salmonid fish populations with one intestinal parasite species are also very rare (Kennedy, pers. comm.). Brook charr in England always harbor at least two intestinal parasites or none at all (Kennedy, 1978b; Kennedy, pers. comm.). Rainbow trout from some British reservoirs and islands may have one species (Kennedy et al., 1991; Kennedy, pers. comm.). Some reports also exist for brown trout from several localities harboring one intestinal helminth species (Kennedy, 1978a; Kennedy et al., 1986, 1991).

The nature of the parasite and host associations in Sweetwater Creek facilitate the study of the various factors, described by Kennedy (1970), that may influence any changes in size and structure of the E. salvelini population. Specifically, how these input, output and control factors and their interaction vary with time may reveal if there is a seasonal pattern in the biology of E. salvelini. Seasonal patterns in the biology of this cestode have not been studied extensively, with most studies being conducted for a few weeks or few months out of the year (MacLulich, 1943; Sandeman and Pippy, 1967; Smith, 1973).

The objective of this study was to investigate whether there was a seasonal pattern in the biology of E. salvelini infecting brook trout and slimy sculpin from Sweetwater Creek, Michigan. Conventionally, measurements of prevalence and mean intensity are analyzed across time to determine if there are seasonal changes in parasite populations. The

specific goals of this study were (1) to determine seasonal changes in prevalence and intensity of E. salvelini infecting brook trout and slimy sculpin; (2) to determine if host size and sex influence the distribution (whether aggregated, even or random) of this parasite within its host; and (3) to determine if any factors (input, output or control) influence seasonal changes in the size and structure of the parasite population.

MATERIALS AND METHODS

Location and Description of Study Site

Sweetwater Creek is in Lake County, approximately 4 km southeast of Branch, Michigan, within the Manistee National Forest (Figure 1). It flows south into the Pere Marquette River which then flows east into Lake Michigan. The Michigan Department of Natural Resources in 1966 described the creek north of Wingleton Road as having colorless and clear water and a rapid current; the bottom is sand and gravel; the surrounding country is hilly and sandy with a mixed forest. Muzzall (1993a) found the maximum width and depth of the creek to be 4.8 m and 0.9 m, respectively. The creek is divided into three sections by 2 culverts that run under Wingleton Road and a railroad track. All samples were taken from an area north of Wingleton Road for approximately 700 m. The creek's distance between Wingleton Road and its eastern most origin is approximately 1.1 km and 1.3 km from Wingleton Road to the Pere Marquette River.

Collection and Examination of Fish

Brook trout and slimy sculpin were collected by electrofishing every 2 months starting in May 1995 through September 1996. Between May 1995 and January 1996 collection of at least 30 brook trout and 15 slimy sculpin was attempted. Fish were transported to the laboratory alive, killed in MS 222 (tricane methane sulphonate) and then examined for Eubothrium salvelini within 72 hours after collection. If fish were not examined within 72 hours they were frozen and examined later. After March 1996 sample size was increased to 60 trout per sampling period and fish were killed in the field and transported to the laboratory in ice and then frozen. Upon examination, total fish length (mm) and sex were determined and recorded. The gastrointestinal tract, gills, eyes, brain,

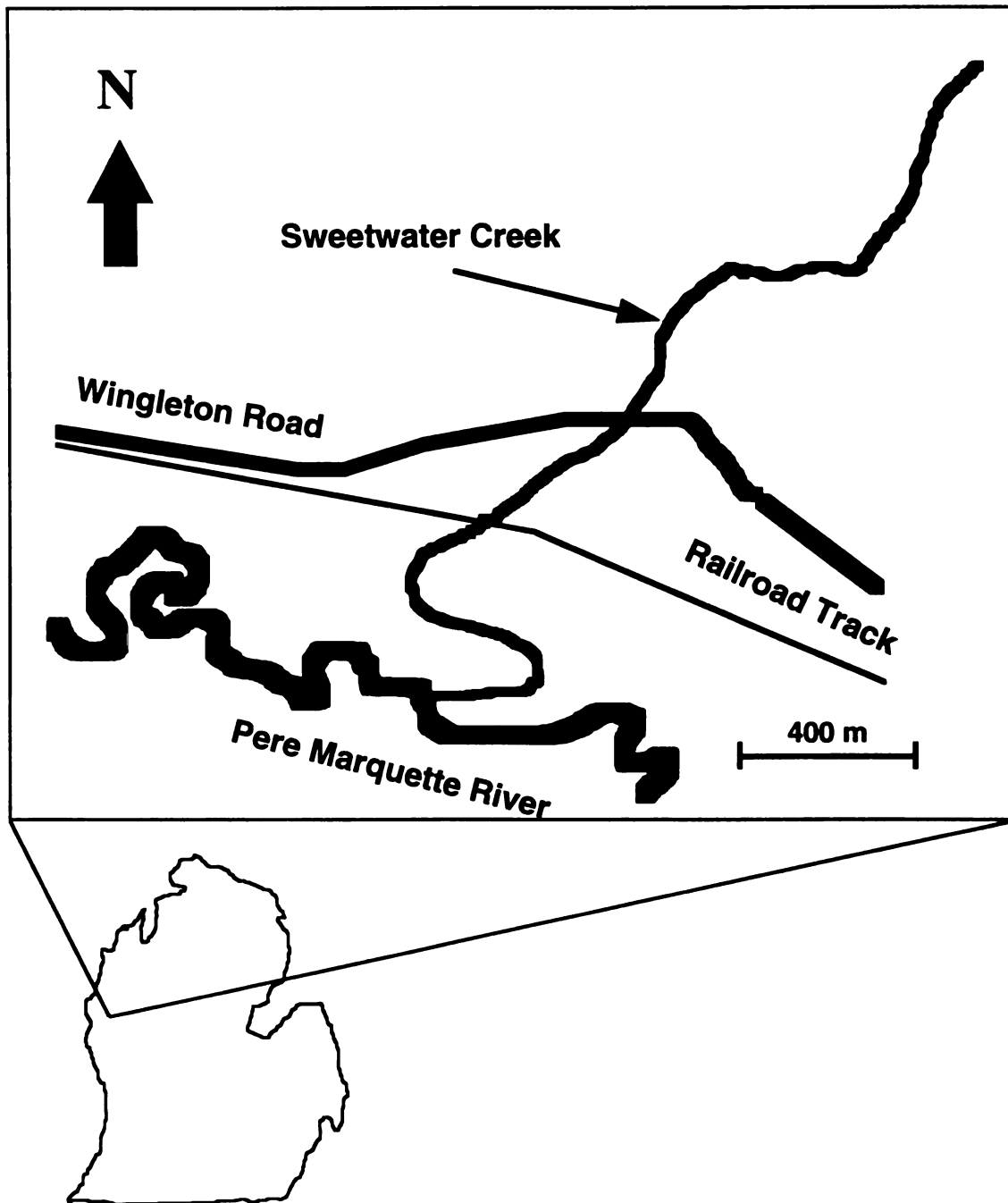


Figure 1. Map of Sweetwater Creek, MI. From Tiger Map Server, U.S. Census Bureau.

esophagus, kidney, liver, gall bladder, spleen, gonads, and musculature of each fish were examined. After March 1996 only the gastrointestinal tract was examined since E. salvelini was the only parasite species found. Parasites were removed, counted, identified and then preserved and stored in 70% alcohol in vials.

Worm Development

Developmental state refers to length (mm), and maturity of each E. salvelini. Descriptions of E. salvelini follow those of Amin (1977) (see also Figure 2). Young adults have a body length of 2.0 to 6.9 mm and a maximum width of 0.4 to 0.7 mm. The scolex is 378 to 560 μm long by 266 to 406 μm wide. The apical disk is not pronounced, its diameter smaller than the widest scolex diameter. The bothria and its pronounced rim occupy slightly more than the anterior half of the scolex; the posterior region is just as wide. The inner margin of the bothria is 154 to 336 μm long by 112 to 168 μm wide. The first segment is 70 to 80 μm long and 350 to 406 μm wide.

Mature adults measure at least 63.8 to 113.8 mm in length. The scolex is elongate with a length of 840 to 1260 μm , a width of 420 to 476 μm and a depth of 406 to 462 μm . The bothria are deep with a length of 448 to 490 μm by a width of 154 to 210 μm at the inner margin and with a strong oval rim in the anterior region of the scolex. The apical disk is conspicuous with a slightly convex apex and deep indentation and at least as wide as the scolex; its diameter is 308 to 518 μm . The posterior region of the scolex is clearly distinct and narrower than the anterior region. The first proglottid is about as wide and deep as the posterior end of the scolex with a length of 140 to 224 μm , a width of 406 to 518 μm and a depth of 322 to 392 μm . The male reproductive system develops first. Fully mature

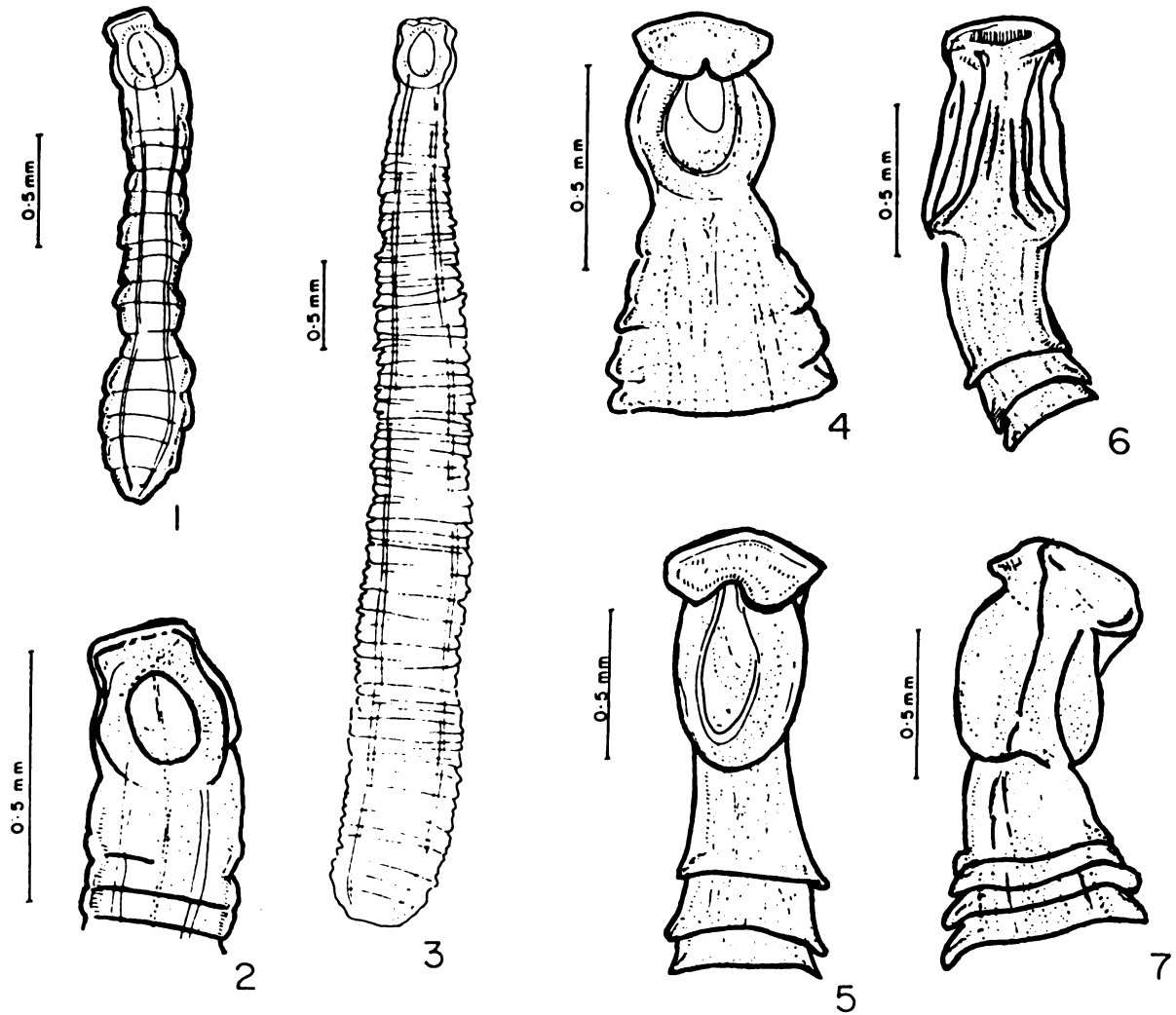


Figure 2. *Eubothrium salvelini* from Lake Michigan fishes, from Amin (1977). 2.1 - Young adult. 2.2 - Scolex of specimen 2.1 enlarged. 2.3 - Larger young adult. 2.4 - Scolex of a large young adult. 2.5 - Scolex of mature adult. 2.6-2.7 - Scolices of two mature adults (lateral view).

proglottids first appear at about 110 mm from the anterior end with 40 to 60 oval testes. Gravid proglottids are 200 to 640 μm long, 1320 to 2920 μm wide and 720 to 1040 μm deep.

Collection and Examination of Fish Stomach Contents

The stomach contents of brook trout captured between March and September 1996 were collected by gastric lavage to determine their diet and if they were feeding on the copepod intermediate host. A 5 cc plastic syringe was fitted with 10 cm of plastic tubing and filled with water. The tube was inserted through the mouth until it reached the stomach of the fish. The syringe was then emptied while the mouth of the fish was placed over a small plastic container to collect the regurgitated stomach contents. The fish were placed in individual bags and the stomach contents were placed in vials, both labeled with matching accession numbers. Once in the laboratory each of the vials was filled with 70% alcohol and stored until examination later. During this period, stomach contents of slimy sculpin were not collected in the field but their stomachs were examined in the laboratory during examination for parasites. Upon examination, food items from each stomach were identified by their common name. For each group of organisms found in the stomach, a percentage of the diet as well as occurrence (percent of fish in which each food type was found) from all fish collected that month were determined. Also, each organism found in the stomach was dissected and examined for the procercoid stage of E. salvelini.

Collection and Examination of Copepods

Copepods were collected approximately every 2 weeks (starting in May through October 1995 and again in May through September 1996) using an aquatic dip net (D-

shaped) lined with 125 μ mesh. The dip net was dragged for approximately 1.5 m through the muddy bottom of littoral zones or pools in the creek since this is the most likely habitat for copepods (Pennak, 1978). The location of collection varied from date to date since the constant change in the creek's current formed and destroyed these areas randomly. Samples taken with the dip net were then passed through a sieve series, with the smallest sieve being 250 μ m, and then brought to the lab in glass jars. Two samples were collected for every sampling date. In the lab the mud samples were preserved and stored in 70% alcohol. Bengal Rose dye was added to the jars to make detection of copepods easier during examination. Each of the jar contents were placed in 20 Petri dishes. Any copepods in the mud stained pink and were picked out. Copepods were then stored in vials for later identification and examination for E. salvelini. Copepods were identified using the keys of Edmondson (1959) and Pennak (1978). Copepods were put on slides in glycerin and examined for E. salvelini procercoids using a compound microscope at a magnification of at least 400X (Boyce, 1974).

Data Analyses

A 2x9 contingency table was used to compare the number of fish infected with E. salvelini to those uninfected between the months sampled. The number of infected versus uninfected male and female fish were compared using Chi-square analysis. This analysis was performed for the overall number of fish collected and between months sampled. A Kruskal-Wallis test was used to compare intensity between months sampled. A Mann-Whitney U test was used to compare intensity between all male and female fish collected. This comparison was also made between different sexes at every month of collection. These analyses were repeated for both brook trout and slimy sculpin independently.

A 2x9 contingency table was used to compare the number of gravid to non-gravid worms between the months sampled. A Kruskal-Wallis test was used to compare the mean length of worms between months sampled. This test was performed independently for worms found in brook trout and slimy sculpin. A Spearman rank correlation was performed to investigate if a correlation existed between number of worms and host length. All tests were performed using SYSTAT 6.0 and significance was set at the 5% level for all analyses. The observed parasite population distribution was determined and compared to the fitted Negative Binomial and Poisson distribution using the ParaDis 1.7 software obtained from the internet at www.bondy.orstom.fr/~pichon. Prevalence is defined as the percentage of fish infected in each sample and intensity is the number of worms per infected fish.

RESULTS

Descriptive Statistics of Brook Trout

A total of 392 brook trout (133 in 1995 and 259 in 1996) was collected and examined for E. salvelini in May 1995 through September 1996. Total length (mm) for all brook trout collected each month is summarized in Appendix A. There was a significant difference in fish length between months (ANOVA, $F = 3.38$, $DF = 8$, $P = 0.0009$). Specifically, the differences were between January and September 1996 and between March and September 1996 because larger fish were not collected in January and March when compared to September 1996. A total of 187 female and 190 male brook trout was examined. Fifteen small fish were not sexed because their gonads were not found.

Eubothrium salvelini in Brook Trout

A total of 74 brook trout (55.6 %) and 113 (43.6 %) was infected with E. salvelini in 1995 and 1996, respectively. No seasonal pattern was evident when prevalence was compared between months ($X^2 = 9.49$, $DF = 8$, $P = 0.303$; Figure 3). Eubothrium salvelini were found in either the pyloric caeca or anterior small intestine. Mean intensity $\pm$ standard deviation and ranges (min - max) for 1995 alone and 1996 alone were 3.19 ± 4.35 (1 - 34), 2.52 ± 2.77 (1 - 19), respectively. There was no significant difference when mean intensity was compared between months (Kruskal-Wallis test = 12.246, $P = 0.142$; Figure 4). There was a significant difference between the observed frequency distribution and the Poisson distribution fitted from the data ($X^2 = 278.46$, $DF = 4$, $P < 0.0001$). However, there was no significant difference between the observed distribution and Negative Binomial distribution fitted from the data using the maximum likelihood

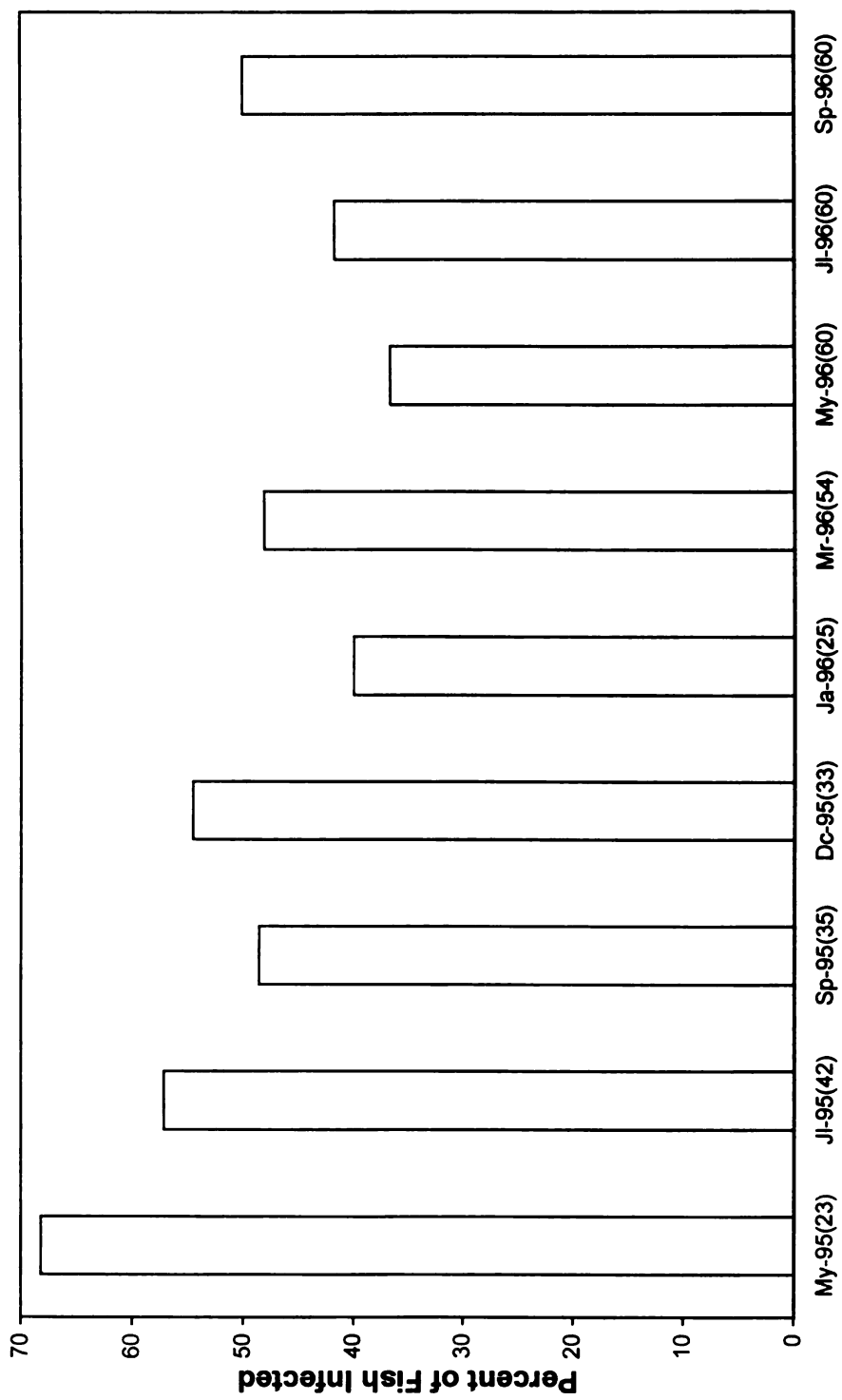


Figure 3. Prevalence of *E. salvelini* from brook trout compared between months sampled. Numbers in parenthesis represent sample size.

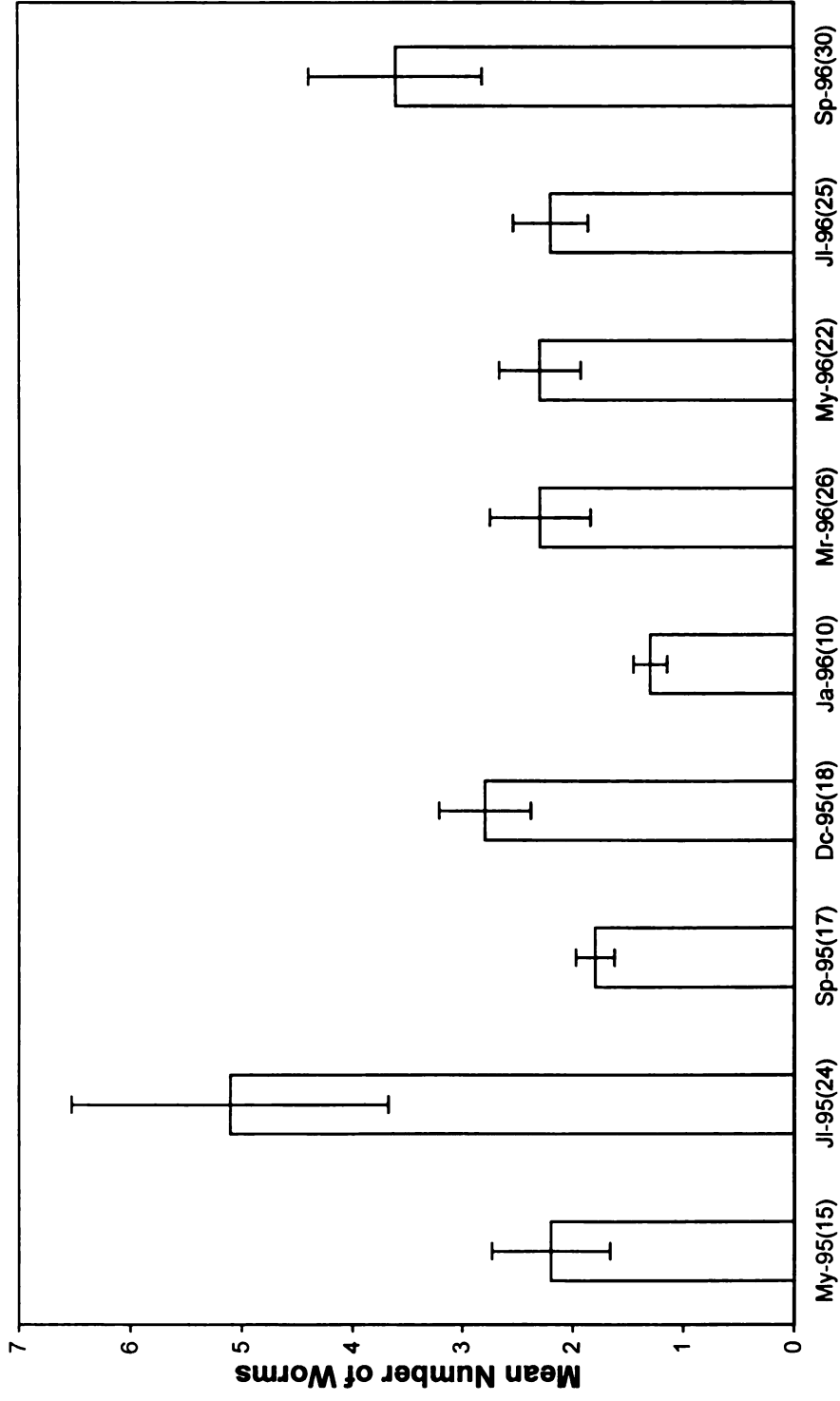


Figure 4. Mean intensity ($\pm$ SEM) of *E. salvelini* from brook trout compared between months sampled. Numbers in parenthesis represent sample size.

k value of 0.45 ($X^2 = 11.55$, $DF = 7$, $P = 0.12$) (Figure 5). The distribution of E. salvelini in brook trout can be described as aggregated and fits a Negative Binomial function.

There was a significant correlation between host length and number of worms in 1995 and 1996 (Spearman Correlation = 0.216, $P < 0.01$). Based on the length of the smallest trout (99 mm) that fed on slimy sculpin, brook trout were separated into 2 length classes; class 1 included fish < 99 mm and class 2 included fish ≥ 99 mm. Thirty nine trout in class 1 in 1995 and 1996 did not show a significant correlation between fish length and number of worms (Spearman Correlation = 0.074, $P > 0.05$), but 148 trout in class 2 did (Spearman Correlation = 0.219, $P < 0.05$). Analysis by year showed that class 1 trout ($n = 18, 21$ for 1995 and 1996, respectively) and class 2 trout ($n = 56, 91$ for 1995 and 1996, respectively) did not show a significant correlation between fish length and number of worms. There was a significant difference in prevalence ($X^2 = 22.137$, $DF = 1$, $P < 0.001$) but not in intensity (Mann-Whitney U test = 2488, $P = 0.1$) between class 1 and class 2 fish.

No significant difference was found between the overall number of infected females and males ($X^2 = 0.21$, $DF = 1$, $P = 0.647$). When the number of infected females and males were compared for each month, a significant difference was found only during March 1996 ($X^2 = 6.07$, $DF = 1$, $P = 0.014$; Figure 6). No significant difference was found when the mean intensity was compared between all females and males (Mann-Whitney U test = 4335, $P = 0.467$) or when females and males are compared each month.

There was a seasonal pattern in the development of E. salvelini in brook trout (Figure 7). A significant difference was found between gravid and non-gravid worms when compared between months ($X^2 = 123.54$, $DF = 8$, $P < 0.00001$). Also, a significant difference was found in mean worm length between months (Kruskal-Wallis test = 102.41, $P < 0.00001$). More gravid and longer worms were found during May of each year, while few gravid and shorter worms were found during September of each year.

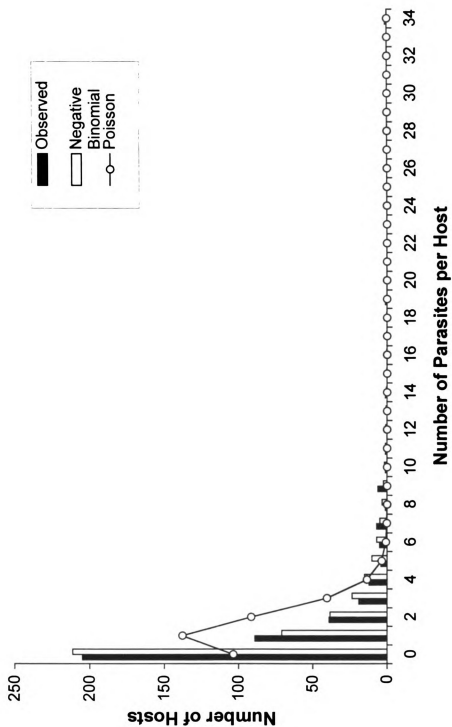


Figure 5. Observed frequency distribution versus the fitted Poisson and Negative Binomial distributions of *E. salvelini* in brook trout.

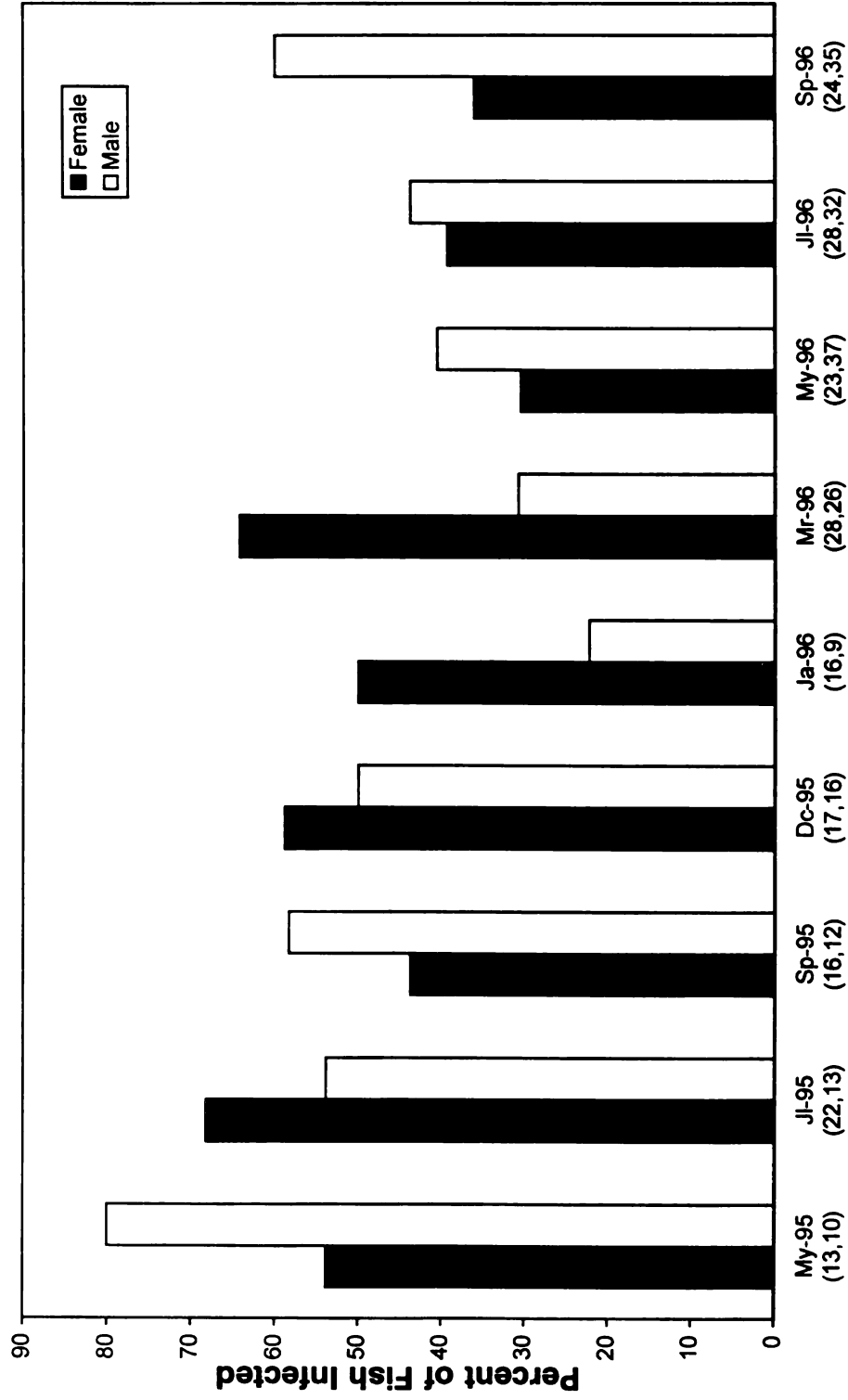


Figure 6. Prevalence of *E. salvelini* in male and female brook trout compared for each month. In parenthesis are sample sizes for females, males.

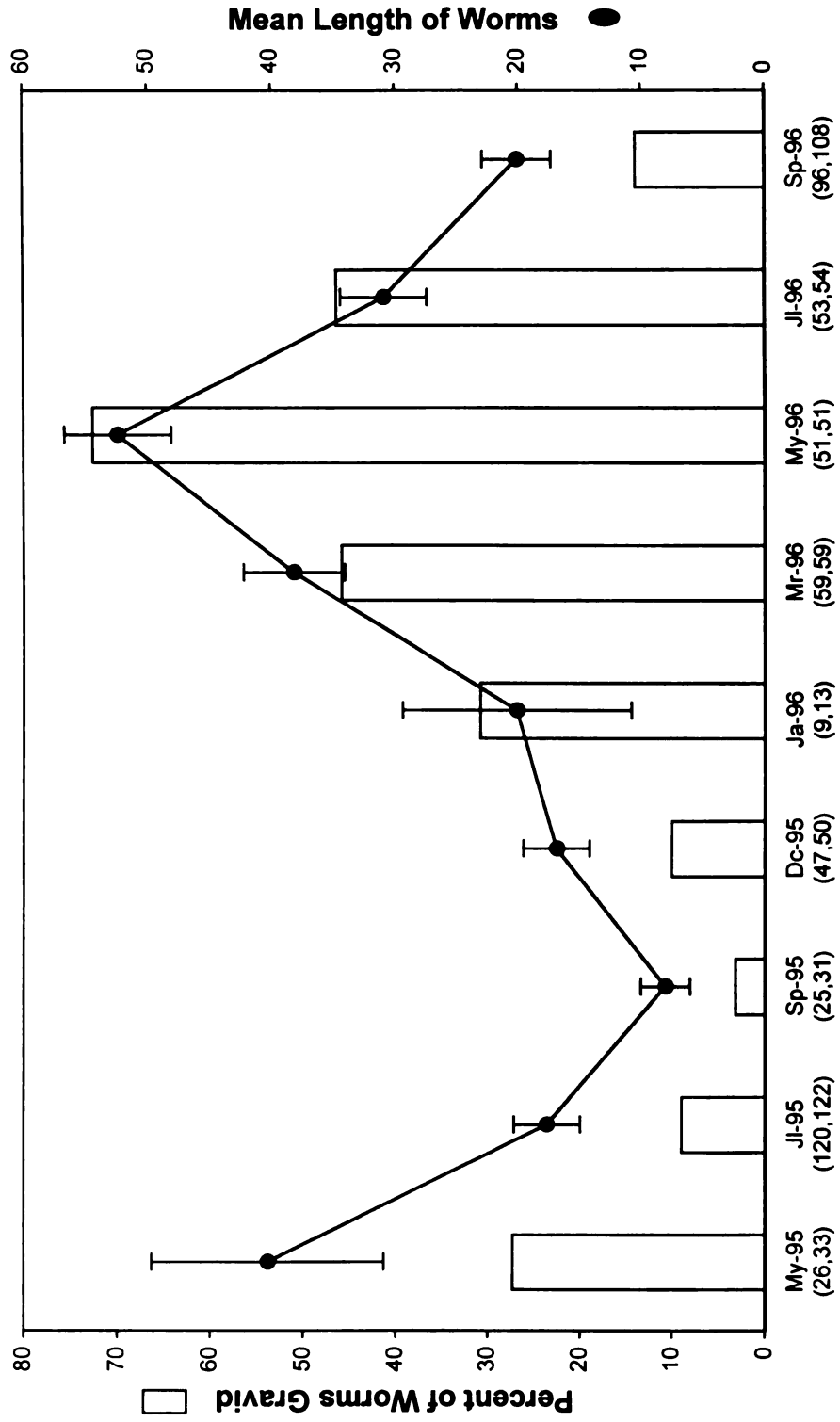


Figure 7. Mean length (mm) ($\pm$ SEM) and percent gravid *E. salvelini* from brook trout compared between months. In parenthesis are sample sizes for length, % gravid.

The stomach contents for brook trout in March through September 1996 are summarized in Table 2 (Appendix B). During March, nearly 80 % of their diet consisted of crane fly adults, midge larvae and stonefly adults. In May, July and September, caddisfly larvae made up the highest percentage of brook trout's diet. Copepods were only found during May and consisted of 0.1 % of the trout's diet. Amphipods and ostracods were found consistently during each month sampled. Seven sculpin were found in the stomach of trout in March, May and September.

Descriptive Statistics of Slimy Sculpin

A total of 211 slimy sculpin (84 in 1995 and 127 in 1996) was examined for E. salvelini in May 1995 through September 1996. Total length (mm) for all slimy sculpin collected each month is summarized in Appendix A. There was no significant difference in fish total length between months. A total of 91 female and 99 male slimy sculpin was examined. Twenty one small fish were not sexed because their gonads were not found.

Eubothrium salvelini in Slimy Sculpin

A total of 23 slimy sculpin (27.4 %) was infected with E. salvelini in 1995 and 13 (10.2 %) was infected 1996. No seasonal pattern was evident when prevalence was compared between months but there was a statistical difference ($X^2 = 26.56$, $DF = 8$, $P = 0.0008$; Figure 8); Pairwise comparisons resulted in differences between May 1995 and all months in 1996, July 1995 and all months in 1996, and December 1995 and all months in 1996. Eubothrium salvelini were found in the small intestine. Mean intensity $\pm$ standard deviation and ranges (min - max) 1995 alone and 1996 alone were 2.04 ± 2.06 (1 - 8) and 1.08 ± 0.28 (1 - 2), respectively. There was no significant difference or seasonal pattern

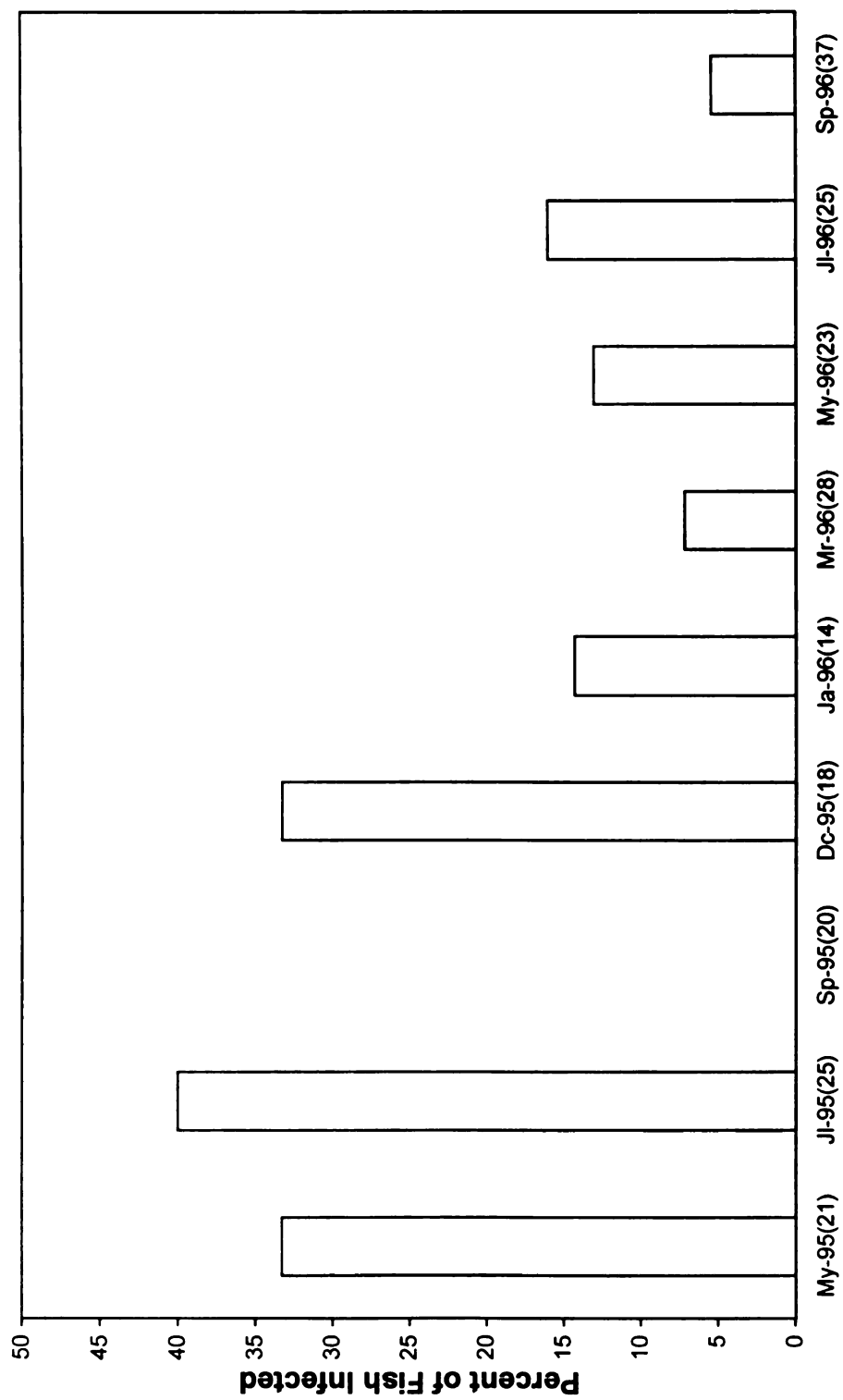


Figure 8. Prevalence of *E. salvelini* from slimy sculpin compared between months sampled. Numbers in parenthesis represent sample size.

when mean intensity was compared between months (Kruskal-Wallis test = 5.23, $P = 0.631$; Figure 9). There was a significant difference between the observed frequency distribution and the Poisson distribution fitted from the data ($X^2 = 9.87$, $DF = 1$, $P = 0.002$). However, there was no significant difference between the observed distribution and Negative Binomial distribution fitted from the data using the maximum likelihood k value of 0.21 ($X^2 = 2.97$, $DF = 2$, $P = 0.23$) (Figure 10). No significant correlation between host length and number of worms existed (Spearman Correlation = 0.13, $P > 0.05$)

No significant difference was found between the overall number of infected females and males ($X^2 = 0.27$, $DF = 1$, $P = 0.602$). When infected females and males were compared for each month no significant differences were found. No significant difference was found when the mean intensity was compared between all females and males (Mann-Whitney U test = 115, $P = 0.355$). When females and males are compared each month a significant difference is found in May and December 1995 ($P = 0.05$ and 0.04 , respectively), however samples sizes consisted of 5 and 6 fish, respectively.

There was no seasonal pattern in the development of E. salvelini in slimy sculpin (Figure 11). Gravid worms were not found in slimy sculpin. There was no significant difference in mean length of worms between months sampled (Kruskal-Wallis test = 1.92, $P = 0.964$).

The stomach contents for slimy sculpin collected during March through September 1996 are summarized in Appendix C. During March, nearly 70 % of the sculpin's diet consisted of ostracods, Cyclops spp. and amphipods. In May, July and September, midge larvae made up the highest percentage of its diet. Copepods were found consistently in all months except September 1996 and amphipods and ostracods were also common in each month.

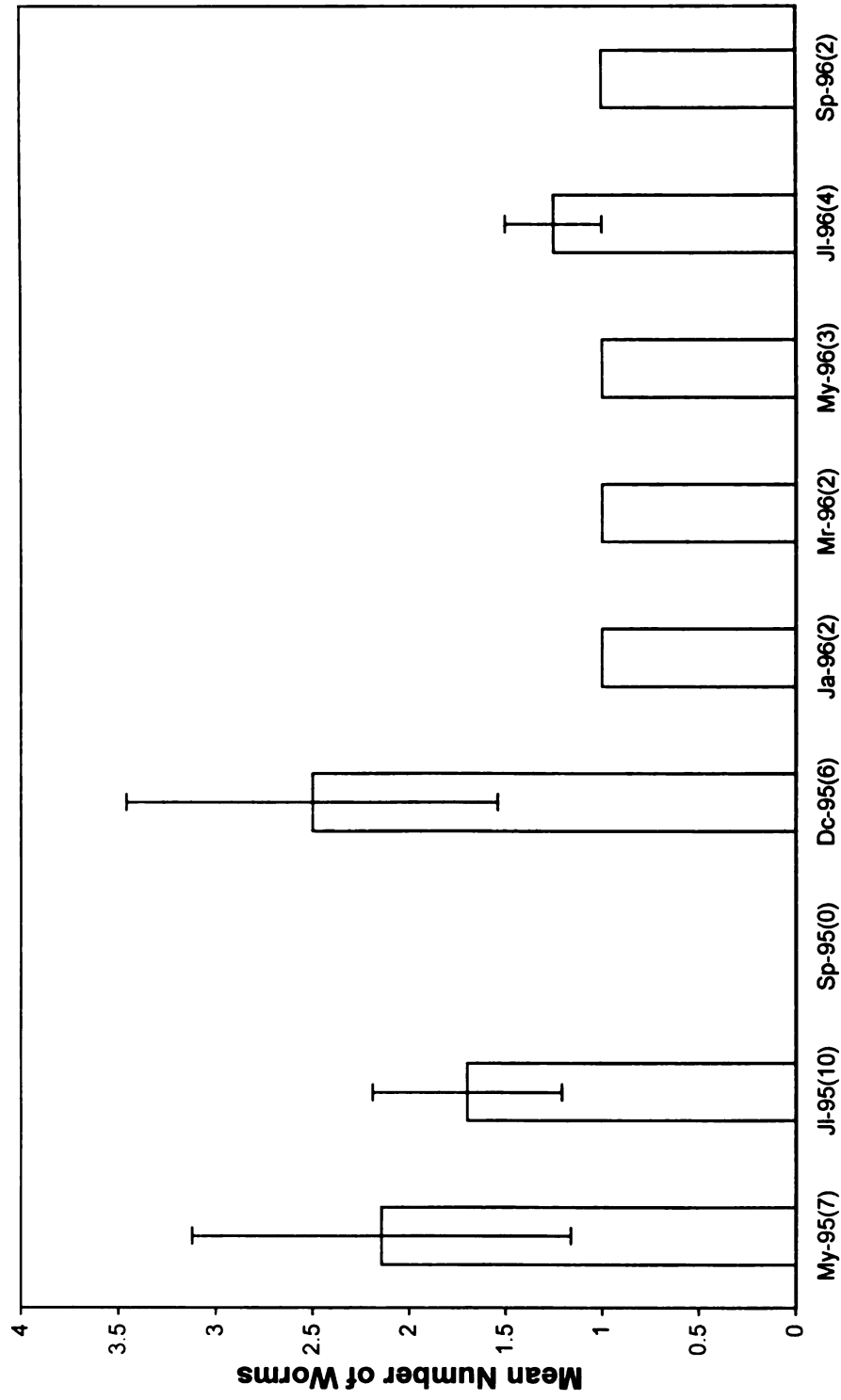


Figure 9. Mean intensity ($\pm$ SEM) of *E. salvelini* from slimy sculpin compared between months sampled. Numbers in parenthesis represent sample size.

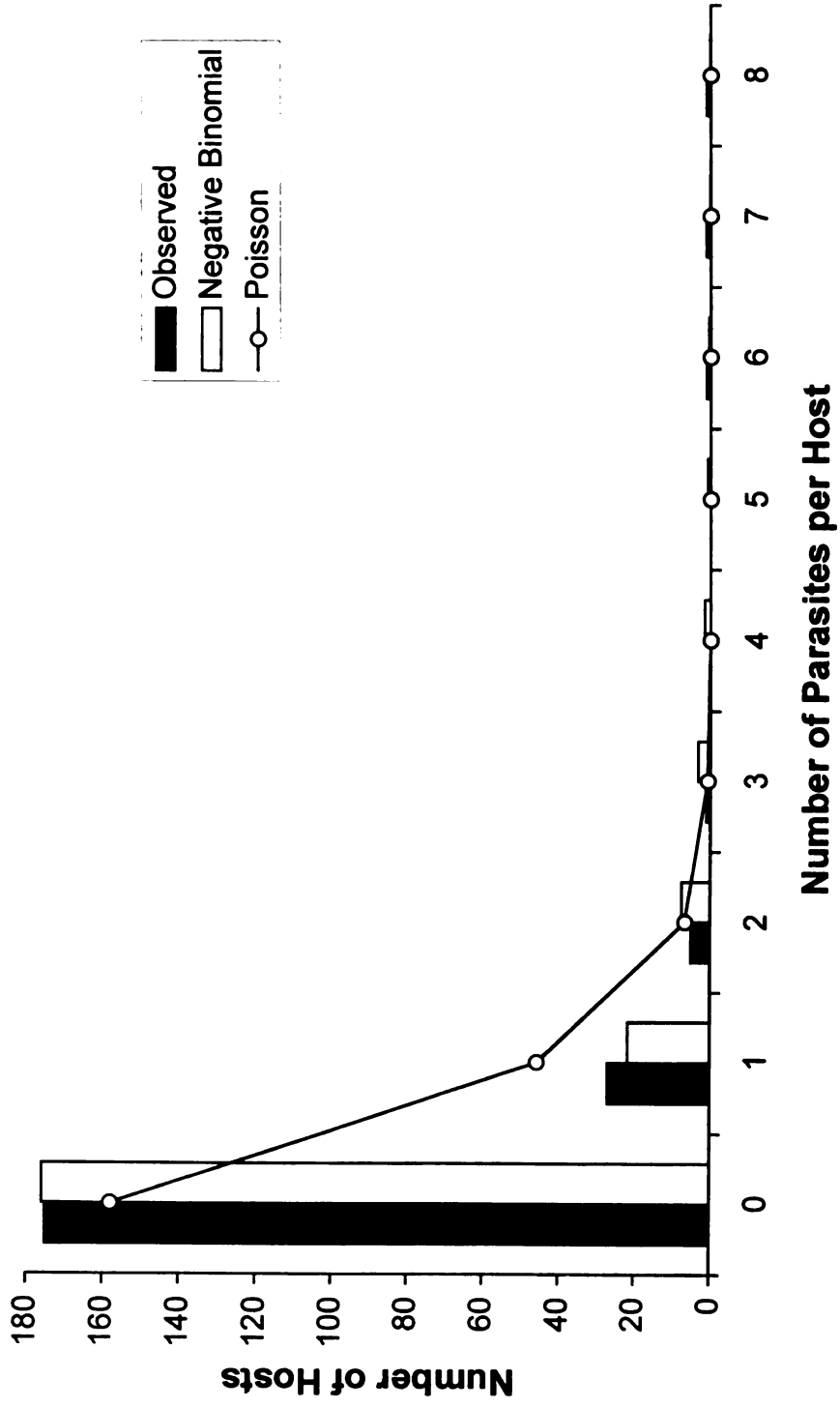


Figure 10. Observed frequency distribution versus the fitted Poisson and Negative Binomial distributions of *E. salvelini* in slimy sculpin.

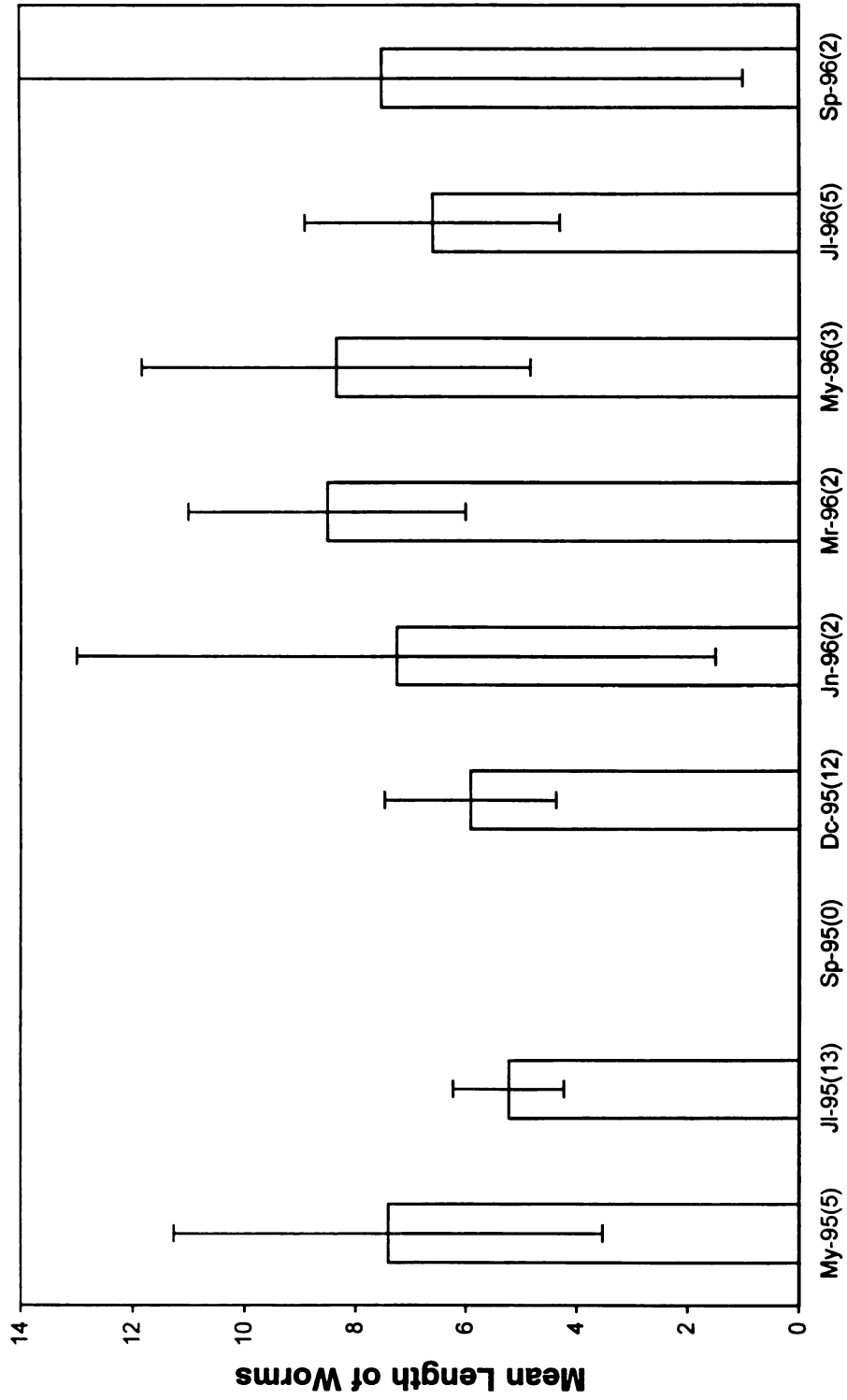


Figure 11. Mean length (mm) ($\pm$ SEM) of *E. salvelini* from slimy sculpin compared between months sampled. Numbers in parenthesis represent sample size.

Identification and Examination of Copepods

The copepods from Sweetwater Creek belong to the suborders Cyclopoida and Harpacticoida. The body of copepods is segmented and is divided into two major regions by an articulation into the anterior region (metasome) and posterior region (urosome). Members of the Cyclopoida have a metasome much wider than the urosome and their first antennae have between 6 and 18 segments that reach from the proximal thirds of the cephalic segment to the end of the metasome. Members of the Harpacticoida have a urosome about as wide as the metasome, both being more or less cylindrical; the first antennae have between 5 and 9 segments that reach from the proximal fifth to the end of the cephalic segment. Cyclopoid females carry 2 egg sacs laterally while Harpacticoid females carry 1 egg sac medially. Specimens of either suborder can be keyed to genus by using some of the characteristics mentioned above but more importantly it can be accomplished by examination of the dissected fifth leg.

Two genera of the Cyclopoida were identified, Cyclops and Tropocyclops. Specimens were identified as follows: both have the fifth leg consisting of 1 or 2 distinct segments and their first antennae have between 6 and 17 segments; Cyclops spp. have 2 distinct segments on their fifth leg with the distal segment of leg 5 small and armed with an apical seta and usually a short or moderately long inner lateral or subapical spine. Tropocyclops spp. have 1 distinct segment on their fifth leg which is broad and armed with an inner spine and 2 outer setae, have first antennae with 12 segments, males and females have their caudal ramus 3 times as long as broad but females' caudal ramus is without spinules (minute spines) on its outer margin.

Only 1 genus of Harpacticoida was identified, Bryocamptus. Specimens were identified as follows: leg 1 had an armed distal exopod with 3 segments and segment 2 had inner setae; the endopod of leg 1 was 3 segmented; females had 7 to 9 segments on their

first antennae; female legs 2 and 3 have 3 segmented endopods while their fifth leg have 5 setae on the basal expansion.

A total of 6399 copepods was examined for E. salvelini proceroids in 1995 and 1996, 3884 in 1995 and 2515 in 1996. A total of 1712 Bryocamptus spp., 1797 Cyclops spp., and 375 Tropocyclops spp. was examined in 1995. In 1996, 1278 Bryocamptus spp., 972 Cyclops spp., and 265 Tropocyclops spp. were examined. No copepods were infected with E. salvelini in 1995 or 1996.

DISCUSSION

A goal of population ecology is to count or estimate the number of organisms in natural populations and try to explain their distribution by (1) counting them at various points in time and (2) relating changes, if any, to biotic and abiotic factors (Anrewartha, 1970; Esch et al., 1977). When studying parasite populations, ecologists must be able to first define what the parasite population is. A parasite population may be the number of individuals of a given species occurring in an individual host (termed the infrapopulation) or all individuals of a given parasite species, in all stages of development, within all hosts of an ecosystem (termed the suprapopulation) (Margolis et al., 1982). When studying free living populations we don't just study one particular age class or stage of a species. Therefore, when studying parasite populations, it is useful to understand what regulates their distribution at both the infra and suprapopulations (Esch et al., 1977).

When studying parasite populations with an indirect life cycle, it is necessary to understand what factors influence any distributional patterns at all stages of the parasite's life cycle. We would therefore study the parasite suprapopulation which is influenced by various factors acting at each of the infrapopulation levels. For example, when studying a population of a parasite species with a 2 host life cycle, we would study any patterns occurring within the first (intermediate) and the second (definitive) host. However, studying the suprapopulation of parasites with such life cycles is difficult because they involve such complex interactions between intermediate and definitive hosts. Also, analyzing the relationship among factors that regulate at the infra and suprapopulation levels is difficult (Esch et al., 1977).

Statistical distributions of parasite and host populations may provide information about each species biology. Models and empirical data have shown that the distribution of parasites is overdispersed or clumped (Kennedy, 1968; Kennedy and Hine, 1969; Crofton, 1971; Anderson, 1974a; Boxhall, 1974; Anderson and May, 1978; May and Anderson,

1978; Anderson and Gordon, 1982). There tends to be large variance to mean ratio in such distributions and this parameter is sometimes used as a measure of dispersion. However, the Negative Binomial is more often used to describe the aggregated nature of parasite distributions (Crofton, 1971), although new measures have been suggested (Poulin, 1993). The k parameter from the Negative Binomial equation $(q - p)^{-k}$ is often used as a measure of aggregation, where the smaller the value of k the more aggregated the distribution is (Anderson and May, 1978; Poulin, 1993). In many parasite-host systems the k value tends to be less than 1.0 (Anderson and May, 1978) but can range between 0.1 and 5 in human helminth infections (Guyatt et al., 1990). The distribution of E. salvelini in brook trout and slimy sculpin is clumped and fits a Negative Binomial (Figures 5 and 10). Maximum likelihood k values are 0.45 and 0.21 for brook trout and slimy sculpin, respectively. Most fish carry no or only a few parasites and only a few are heavily infected. Heterogeneity (differences in the hosts that influence the distribution of the parasites) in the rate of gain or loss of parasites among hosts is thought to be the main factor that increases aggregation (Anderson, 1982). Heterogeneity may be a consequence of differences in age or sex of the host, differences in the history of past exposure to infection, differences in contact between parasite infective stages and hosts due to differences in host behavior, and genetic differences in susceptibility to infection (Scott, 1987). Also, heterogeneities may be due to changes in climate over time or space (Anderson and May, 1979).

Fish sex and size have been hypothesized as possible factors influencing such parasite aggregations (Dogiel et al., 1961; Kennedy, 1970; Esch et al., 1977). Pailing (1965) demonstrated that male Windmere trout between 5 and 7 years old consistently harbored more Discocotyle sagittata, a monogenetic parasite, than females. Kennedy (1968) found that the cestode Caryophyllaeus laticeps infected female fish more heavily for a short time of the year; mainly the breeding season of the fish. The clumped distribution of E. salvelini can not be explained in relation to fish sex since no differences were found

between the number of female and male brook trout infected with E. salvelini. Even when the number of male and female brook trout infected were analyzed for each collection month independently, only a significant difference was found during March 1996 (Figure 6). No biological explanation for this difference can be postulated. Similar results were found for slimy sculpin.

Anderson (1974b) showed that fish size, and therefore age, was closely related to the number of Diplozoon paradoxum, a monogenean on the gills of fish. A significant correlation between host length and number of E. salvelini was found in brook trout. Stomach analyses showed that brook trout fed on slimy sculpin in 1996 (Appendix B) and that the smallest trout that fed on sculpin was 99 mm. When trout were grouped into 2 length classes based on this measure, a significant difference in prevalence was found between large and small fish but no difference was found in intensity. Therefore, large fish are more frequently found infected with E. salvelini than are small fish. A significant positive correlation between length and number of worms was also found in large but not small fish. This correlation, along with differences in prevalence, may be factors that can explain the clumped distribution of E. salvelini in brook trout. Feeding on infected slimy sculpin by large brook trout can contribute to the observed clumped distribution and therefore differences in feeding behavior by hosts may be a factor explaining the observed distribution. In slimy sculpin the observed distribution is also significantly different from the fitted Poisson distribution and fits a Negative Binomial which suggests that E. salvelini is clumped in this host species too (Figure 10). However, fish length can not be a factor explaining this distribution because no significant correlation between host length and number of E. salvelini in slimy sculpin was found for the entire sampling period. No biological explanation for the aggregated distribution of E. salvelini in slimy sculpin can be formulated at this time.

How parasite populations vary in time has been a subject extensively studied and usually measured in terms of differences in prevalence and intensity (Kennedy, 1970). In

fish parasite population studies, seasonal cycles have been described for prevalences and intensities of monogeneans, trematodes, acanthocephalans, copepods and leeches (see Esch et al., 1977 and references there in). In cestodes, a seasonal pattern has been shown for several species as well (Hopkins, 1959; Chubb, 1963; Kennedy and Hine, 1969; Anderson, 1974a; Riggs and Esch, 1987; Margolis and Esch, 1989). No studies of this kind have been conducted for E. salvelini, but studies have been done for E. crassum infecting brown trout from a small British lake (Kennedy, 1996). Kennedy's study found that a new generation of parasites infect trout in Spring and Summer based on the high prevalence and abundance values and small size of the majority of the parasites during these times. Prevalence and intensity of E. salvelini in brook trout (Figures 3 and 4, respectively) and slimy sculpin (Figures 8 and 9, respectively) did not significantly vary with time in this study. The results demonstrate that the parasite is present all year long but do not indicate whether recruitment is seasonal or not. A seasonal pattern might be revealed if we study what factors (e.g. input, output and control as described by Kennedy (1970)) may regulate the changes in the size and structure of the parasite population.

Input factors

One input factor that may influence changes in the size and structure of the parasite population is the availability of infective larvae in the environment. The life cycle of E. salvelini involves a copepod intermediate host which harbors the infective larvae and Cyclops spp. have been identified as intermediate hosts in lakes (Vik, 1963; Smith, 1973; Boyce, 1974). Muzzall (1993a) suggested that E. salvelini was present in resident brook trout from Sweetwater Creek and adult salmonids from Lake Michigan, but not in young salmonids from the Pere Marquette River because the intermediate hosts must be absent in the latter. My study confirms the suggestion by Muzzall (1994a) that copepods are present in Sweetwater Creek. However, copepods were not infected. It is very likely that this

could have been due to a variety of factors. First, the collection technique in this study was not very quantitative regarding number of copepods collected or exhaustive. The D-shape dip net was dragged through only 1.5 m of muddy bottom in the littoral zones of the creek and only one of these zones (seldom the same one) was sampled every two weeks. However, this could only be controlled for if every muddy littoral zone was sampled from the entire creek every 2 weeks. Clearly this is nearly impossible since the time and amount of people to conduct such an exhaustive survey of copepods would be enormous. In addition, given the dynamics of the creek, the number and location of the littoral zones would be hard to consistently sample because they vary over time and season. Even in studies done in lakes, which are ecosystems that are better contained than are rivers and streams, 2 out of approximately 6200 copepods were found to be infected with E. salvelini (see Boyce, 1974). Therefore, the probability of finding an infected copepod in Sweetwater Creek was very low to begin with.

Although a direct measure of the availability of E. salvelini infective stages in the environment was not possible, an indirect one is. When we look at the mean length of E. salvelini infecting brook trout (Figure 7), we see that a seasonal pattern in maturation is present. The longest worms occur during May of each year, while the shortest worms occur during September of each year. Similarly, the percent of gravid worms follows the same seasonal pattern, with most gravid worms occurring during May of each year while the smallest number of gravid worms are found during September of each year. Sandman and Pippy (1967) examined several trout populations and found E. salvelini to vary in their maturity with season. Samples taken during the spring and early summer months contained mature cestodes while samples taken in late August, September or later contained immature specimens only. These authors suggested that fish were becoming infected during August and that the parasites matured during winter and spring, released their eggs, and died in late summer. Similarly, my results suggest that in brook trout, recruitment is occurring in mid Summer to early Fall and that the worms mature over the Winter.

No gravid worms were found in sculpin and no seasonal pattern was found in the length of worms (Figure 11). Because E. salvelini does not mature in this fish species, it is suggested that sculpins are either a transport (paratenic) host or a dead end host. A paratenic host is a host in which the parasite does not undergo any development but in which it remains alive and infective to another host, bridging an ecological gap between intermediate and definitive hosts (Roberts and Janovy Jr., 1996). In order to determine whether E. salvelini may be using slimy sculpin as a paratenic host direct evidence is needed. Studies on other cestodes with a life cycle similar to E. salvelini have shown that in addition to copepods serving as the intermediate host, young infected fish could serve as a paratenic host. Wagner (1954) experimentally studied the life history of Proteocephalus tumidocollus in rainbow trout reared in outdoor enclosures. He concluded that although several species of copepods served as intermediate hosts and no second intermediate host was required for the completion of the parasite life cycle, young infected trout fed to larger trout of the same species could successfully transfer the parasite from fish to fish. It is possible that this may also be occurring with E. salvelini in Sweetwater Creek when brook trout feed on infected slimy sculpin. This could also be the case of large brook trout that feed on smaller infected brook trout.

A second input factor that may regulate the flow of parasites through fish is variation in the diet and feeding habits. Intestinal helminth species composition in fish (and other vertebrates) is predicted to vary with diet and vagility (Kennedy et al., 1986). Although adult salmonids are generalist feeders, they go through an ontogenetic change in diet as do most other fish (Wootton, 1990). Young brook trout feed primarily on small invertebrates while adults can feed on larger insects and fish (Power, 1980). For this reason one might predict that the number of helminths infecting fish will increase with host age because longer, older host will have been exposed to infective stages for a longer period of time and have a more varied diet. Seven slimy sculpin were found in the stomachs of seven brook trout and the smallest fish that fed on sculpin was 99 mm. My

study found a significant correlation between the number of E. salvelini and host length in those fish larger than 99 mm. This provides evidence that larger trout in this creek feed on sculpin and suggests that transfer of E. salvelini from sculpin to trout is possible. Also, it partially explains why the parasite distribution is clumped with most E. salvelini found in large piscivorous trout.

Stomach content analyses for brook trout showed that they only fed on copepods during May 1996 (Appendix B). Other crustaceans (ostracods and amphipods) which are more closely related to copepods than any other food item were found more abundantly and frequently than were copepods. All invertebrates (and occasional vertebrates) in the stomachs were examined for the infective stages of E. salvelini and none were infected. Also, stomach content analyses show that sculpin were feeding on copepods more often than were trout (Appendix C). Therefore, the probability of feeding on an infective copepod appears to be greater in the sculpin than trout. Since there is a significant correlation between brook trout length (≥ 99 mm) and the number of worms, this provides further explanation for the clumped distribution of E. salvelini in trout (i.e. fewer worms are found in the smaller trout).

Resident fish will have a more restricted diet than fish that can move across different habitats which will potentially expose them to a more varied parasite fauna (Kennedy et al., 1986). Although stomach contents show that brook trout are feeding on a variety of invertebrates which can potentially serve as intermediate hosts for a variety of parasite species, no other intestinal parasite is present in this area of Sweetwater Creek. This may provide evidence that this is indeed a resident and somewhat isolated population of brook trout. Furthermore, Muzzall (1993b) suggested that no other salmonids are immigrating to this part of Sweetwater Creek and introducing other parasite species. Also, although invertebrates that can serve as intermediate hosts to other parasite species are present (e.g. amphipods, ostracods, mayflies), these might not be present in large enough

numbers for transmission to occur. This could be explained by the low productivity typical of first order creeks (Reid, 1961), such as Sweetwater Creek.

Output factors

Output factors may be events such as the failure of the parasite to establish themselves, rejection of the parasite by the host, and parasite senescence. The first two factors may be influenced by the immune response of the host. Host immune responses in regulating the size and structure of parasite populations have been extensively studied in mammalian parasite systems. In fish, however, the role of the immune response is still not well understood. There is not much evidence to suggest that fish can and do prevent the establishment of parasites in their body although the role of the mucus secretions on the skin have been suggested to be one possible response (Kennedy, 1977). Senescence of the parasite is an important output factor. Sandeman and Pippy (1967) suggested that E. salvelini have a longevity of approximately 1 year, while Smith (1973) suggests it lives for almost 2 years. Although this study did not measure the longevity of E. salvelini, it might be suggested that it lives for approximately 1 year based on the seasonal pattern in the length and percent gravid worms infecting brook trout (Figure 7).

Control factors

Interspecific competition can be ruled out as a possible control factor influencing the size and structure of the E. salvelini population in both brook trout and slimy sculpin since only this intestinal parasite exists in this part of Sweetwater Creek. Intraspecific competition may not be playing an important role either. These worms might possibly compete for space, but the overall mean intensity of infection in both fish species is low (2.79 in brook trout and 1.69 in slimy sculpin) compared to the number of pyloric caeca in

brook trout (23 - 55) (Page and Burr, 1991). Therefore space does not appear to be a limiting factor. Nutrients might possibly regulate the infrapopulation size but few studies have shown direct evidence on what the food resources are for most parasites (but see Bansemir and Sukhdeo, 1994).

Water temperature has been suggested as a possible control factor regulating the size and structure of fish parasite populations (Esch et al., 1977; Kennedy, 1977). The possible role of this abiotic factor in regulating the population of E. salvelini in Sweetwater Creek is very minimal. Sweetwater Creek is a spring fed first order stream whose water temperature does not fluctuate dramatically throughout the year.

CONCLUSIONS

It is generally accepted that patterns in the size and structure of populations of parasite species with an indirect life cycle can be regulated by variation in ecological factors such as trophic interactions between various animals that may serve as intermediate or definitive hosts, the distribution of these hosts, and the time of the year that samples from the host population are taken (Dogiel, 1962; Esch, 1971). Because of the complexity of these trophic interactions, parasite suprapopulation studies can be difficult to conduct and most studies are done at the infrapopulation level. Prevalence and intensity have traditionally been used to measure seasonal changes in the size and structure of parasite populations and although they are still implemented, the use of input, output and control factors has also been proposed as a method of studying these seasonal changes. The distribution of parasite populations tends to be aggregated and the possible causes of this can also be studied.

I can conclude from this study that the distribution of E. salvelini in brook trout is aggregated but this is not influenced by host sex. However, a positive correlation between host length and the number of worms in large piscivorous fish can be one factor that explains this aggregation. Prevalences and intensities of E. salvelini in brook trout and slimy sculpin did not vary significantly with time. It was not possible to determine whether all factors (input, output and control) regulate seasonal changes in the size and structure of the parasite population. Output factors cannot be measured directly due to the lack of information on the fish immune response and because the longevity of the worm is not known. Control factors do not appear to be important in this system because no other helminth species occur for interspecific competition to play a role, intraspecific competition might not be important since space is not a limiting factor, and water temperature does not vary. However, an attempt at measuring some input factors was possible. Gravid worms were not found in slimy sculpin which suggests that this is a paratenic or dead end host.

Based on the differences in mean worm length and percent gravid worms in brook trout across months, recruitment of E. salvelini by this host appears to occur during late Spring to early Fall. Therefore, it is during this time that infective larvae would be available in the environment for brook trout to recruit new worms, although no copepods were found infected with this stage. Also, stomach analyses showed that copepods were a low percentage of brook trout diet but a higher percentage in slimy sculpin. Because large trout feed on sculpin more often than they do on copepods and because sculpin harbor non mature E. salvelini, it is suggested that the role of slimy sculpin as a paratenic host is possible and important in partially explaining the aggregated distribution of this parasite population.

APPENDICES

APPENDIX A

Table 1. Mean brook trout and slimy sculpin length (mm) and ranges for months sampled.

	Brook Trout	Slimy Sculpin
	n, length $\pm$ SD, (min-max)	n, length $\pm$ SD, (min-max)
May – 1995	23, 117.8 $\pm$ 27, (79 - 173)	21, 64.5 $\pm$ 18, (40 - 97)
July – 1995	42, 107.4 $\pm$ 41, (45 - 200)	25, 65.2 $\pm$ 16, (43 - 96)
September – 1995	35, 110.4 $\pm$ 41, (60 - 210)	20, 68.2 $\pm$ 16, (30 - 101)
December – 1995	33, 119.5 $\pm$ 32, (60 - 192)	18, 75.2 $\pm$ 11, (48 - 91)
January – 1996	25, 99.4 $\pm$ 22, (68 - 151)	14, 72.1 $\pm$ 10, (56 - 88)
March – 1996	54, 102.1 $\pm$ 25, (65 - 156)	28, 69.2 $\pm$ 16, (34 - 93)
May – 1996	60, 113.9 $\pm$ 25, (47 - 182)	23, 68.7 $\pm$ 12, (50 - 94)
July – 1996	60, 111.6 $\pm$ 22, (56 - 165)	25, 76.9 $\pm$ 12, (59 - 100)
September – 1996	60, 124.8 $\pm$ 24, (70 - 182)	37, 66.4 $\pm$ 15, (26 - 95)

APPENDIX B

Table 2. Prey's percent diet composition (% Diet) and frequency of occurrence (% Freq.) in 53, 60, 60 and 60 brook trout collected in March, May, July and September 1996, respectively. Numbers in parentheses are the number of individual prey counted in stomachs (for % Diet) and number of fish in which prey type was found (for % Freq.).

Prey	March			May			July			September		
	% Diet	% Freq.		% Diet	% Freq.		% Diet	% Freq.		% Diet	% Freq.	
Cranefly adults	46.3 (429)	45.3 (24)		-	-		0.2 (1)	1.7 (1)		-	-	
Midge larvae	20.5 (190)	56.6 (30)		25.1 (165)	59.6 (34)		4.2 (22)	30.0 (18)		4.8 (13)	20 (11)	
Stonefly adults	10.0 (93)	22.6 (12)		9.9 (65)	21.0 (12)		0.4 (2)	3.3 (2)		-	-	
Caddisfly nymphs	8.9 (83)	37.7 (20)		26.6 (175)	85.9 (49)		63.6 (329)	90.0 (54)		47.2 (126)	78.2 (43)	
Stonefly nymphs	3.7 (34)	37.7 (20)		1.1 (7)	8.8 (5)		1.0 (5)	6.7 (4)		1.1 (3)	3.6 (2)	
Mayfly nymphs	2.5 (23)	32.1 (17)		0.9 (6)	7.0 (4)		1.2 (6)	6.7 (4)		1.9 (5)	7.3 (4)	
Blackfly larvae	2.0 (19)	9.4 (5)		3.8 (25)	29.8 (17)		7.0 (36)	35.0 (21)		2.6 (7)	11.0 (6)	
Water mites	1.2 (11)	16.9 (9)		2.6 (17)	24.6 (14)		11.9 (62)	45.0 (27)		3.0 (8)	11.0 (6)	
Amphipods	1.2 (11)	9.4 (5)		1.5 (10)	15.8 (9)		1.5 (8)	10.0 (6)		16.5 (44)	32.7 (18)	
Other Diptera	1.2 (11)	3.8 (2)		17.7 (116)	35.1 (20)		1.9 (10)	10.0 (6)		10.5 (28)	14.5 (8)	
Spiders	0.4 (4)	5.7 (3)		0.1 (1)	1.7 (1)		0.4 (2)	3.3 (2)		0.7 (2)	3.6 (2)	
Slimy sculpin	0.4 (4)	7.5 (4)		0.1 (1)	1.7 (1)		-	-		0.7 (2)	0.7 (2)	
Ostracods	0.2 (2)	1.9 (1)		0.6 (4)	3.5 (2)		0.2 (1)	1.7 (1)		0.4 (1)	1.8 (1)	
Springtails	0.2 (2)	3.8 (2)		0.1 (1)	1.7 (1)		-	-		0.4 (1)	1.8 (1)	
Dragonfly nymphs	0.2 (2)	3.8 (2)		-	-		-	-		-	-	
Damselfly adults	0.2 (2)	3.8 (2)		-	-		-	-		-	-	
Beetle adults	0.2 (2)	1.9 (1)		2.7 (18)	17.5 (10)		1.7 (9)	13.3 (8)		2.2 (6)	11.0 (6)	
Beetle larvae	0.1 (1)	1.9 (1)		1.7 (11)	15.8 (9)		-	-		3.0 (8)	14.5 (8)	
Dragonfly adult	0.1 (1)	1.9 (1)		-	-		-	-		-	-	
Moth larva	0.1 (1)	1.9 (1)		-	-		-	-		-	-	

Table 2. (cont'd).

Prey	March		May		July		September	
	% Diet	% Freq.	% Diet	% Freq.	% Diet	% Freq.	% Diet	% Freq.
Mosquito larva	0.1 (1)	1.9 (1)	-	-	-	-	-	-
Earthworms	0.1 (1)	1.9 (1)	0.3 (2)	3.5 (2)	0.6 (3)	5.0 (3)	0.4 (1)	1.8 (1)
Ants	-	-	-	-	0.4 (2)	3.3 (2)	0.7 (2)	3.6 (2)
Hoppers	-	-	-	-	1.7 (9)	13.3 (8)	-	-
Mayfly adult	-	-	0.1 (1)	1.7 (1)	-	-	-	-
Lepidoptera larvae	-	-	-	-	-	-	1.9 (5)	7.3 (4)
Caddisfly adults	-	-	0.3 (2)	3.5 (2)	0.2 (1)	1.7 (1)	-	-
Snails	-	-	2.7 (18)	14.0 (8)	1.0 (5)	8.3 (5)	-	-
Cranefly nymphs	-	-	0.8 (5)	7.0 (4)	-	-	-	-
Millipedes	-	-	0.5 (3)	5.3 (3)	0.2 (1)	1.7 (1)	0.4 (1)	1.8 (1)
Nematodes	-	-	0.5 (3)	1.7 (1)	0.6 (3)	5.0 (3)	0.4 (1)	1.8 (1)
Salamanders	-	-	-	-	-	-	0.7 (2)	3.6 (2)
Other fish	-	-	-	-	-	-	0.4 (1)	1.8 (1)
Copepod	-	-	0.1 (1)	1.7 (1)	-	-	-	-

APPENDIX C

LIST OF REFERENCES

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