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A Comparative Study of the Helminth Fauna of Salmo Gairdneri Rich. From Three Different Environments: A Hatchery, a Lake and, a River

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A COMPARATIVE STUDY OF THE HELMINTH FAUNA OF SALMO GAIRDNERI RICH. FROM THREE DIFFERENT ENVIRONMENTS: A HATCHERY, A LAKE AND, A RIVER

by
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A Thesis
Submitted to the
Faculty of the Graduate College
in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
August 1971

AKNOWLEDGMENTS

The author wishes to express his thanks to everyone, who in many ways helped to prepare this work. To the late Dr. Harold W. Manter and to Mrs. Mary Lou Pritchard for their assistance with the digenetic trematodes, as well as for their kind reception in their laboratory at the University of Nebraska. To Dr. Brent B. Nickol, also of the University of Nebraska for the identification of the acanthocephalans and for the valuable suggestions concerning the literature of the group. To the Biologist in Charge of the Wolf Lake State Fish Hatchery, Mr. James Copeland, as well to all the personnel who always were of great help. To the Michigan Department of Natural Resources for all the help it gave to the project. To the Fisheries Division of the Nebraska Game and Parks Commission in the person of Mr. Glen R. Foster for the first hatchery trout he made available from the Gretna State Fish Hatchery in Nebraska. To Drs. Joseph Engemann, Richard Brewer and Edwin Steen for the constructive ideas given throughout the project. To my wife Suzana for the graphs and constant encouragement.

Jose Felipe Ribeiro Amato

MASTERS THESIS M-3190

AMATO, Jose Felipe Ribeiro

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Western Michigan University, M.A., 1971
Zoology

University Microfilms, A XEROX Company, Ann Arbor, Michigan

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INTRODUCTION

Like all organisms, fish are infested with parasites. The study of these parasites has received much attention in many countries. As in every science, early and some of the present work in fish parasitology, has been devoted to the necessary task of describing the parasites which happen to be found in fish. Parallel to the descriptions of new species, many authors have reported data on the biology of parasites such as new hosts, life cycles and area of distribution.

The Russian parasitologist V. A. Dogiel in 1927 laid the foundations of a branch of parasitology generally known as ecological, where the overall number of parasite species of a given body of water is taken into consideration. The main points investigated in this ecological approach of fish parasitology are the changes in the parasite fauna with the age of the fish host as well as its dependence on the mode of feeding and the diet of the host, the season of the year, the migrations of the host and a number of other factors (Dogiel *et al.*, 1953).

In spite of the relatively early beginning of the ecological approach to fish parasitology in U. S. S. R., much of the early work elsewhere listed new individual forms and did not consider the parasite fauna as a whole.

A difficulty which often arises is that many fish hatcheries used for such studies are only in part available and sometimes out of the surveyor's control. More parasitological studies are needed in which a complete set of data for a body of water is taken at the same time. Comparison of data from a range of waters also seems an effective way of gaining further insight into significant aspects of the biology of fish parasites.

The Problem

The main objectives of this project are

a. to compare the helminthological fauna of rainbow trout *Salmo gairdneri* Richardson at Wolf Lake State Fish Hatchery, Wolf Lake, and Manistee River,

b. to discover if parasites harmful to man infest rainbow trout in these areas of the State of Michigan,

c. to record the incidence and the intensity of helminth parasites in the three different environments, and

d. to record if there is a relationship between the fauna of parasites and the age of the fish hosts.

The Status of the Rainbow Trout

Salmo gairdneri Rich. is the designation used in current work on rainbow trout. *S. irideus* is a synonym found in some older literature. Their place of origin was small brooks of the Coast Ranges of California (Jordan and Evermann, 1896-1900). They have been widely introduced elsewhere. The anadromous (steelhead) and the freshwater (rainbow) types are sometimes put in separate subspecies Salmo gairdneri gairdneri and Salmo gairdnerii irideus respectively (Briggs, 1953; Shapovalov and Taft, 1954; Hildebrand, 1955; Sterba, 1963; Hubbs and Lagler, 1967).

I will use throughout this paper, the name Salmo gairdneri Rich. as it is most often recognized. I will refer to all trout in this study as rainbow trout, even to those collected in the Manistee River, which come from Lake Michigan to spawn in the headwaters of the river.

LITERATURE REVIEW

Many authors have been studying the whole complex of the helminthological fauna of fishes in a given body of water. Some of the authors who at least on some occasion contributed to this field in a general manner are Van Cleave, Bangham, Mueller, Hargis, Fischthal, Hunter, Hoffman and many others in North America and Dogiel, Petrushevski, Travassos, Baer, Skrjabin and Yamaguti from abroad.

More specifically working with monogenetic trematodes in North America are Mizelle, Price and others. From abroad it is possible to cite Bychowski, Malmberg and others. Working with digenetic trematodes in North America are Manter, Pritchard, Stunkard, Cable and many others. Contributions to the acanthocephalans were among others done by Van Cleave, Bangham, Fischthal, Bullock, Nickol, Petroshenko, Yamaguti and many more.

The control of monogenetic trematodes was studied by Moore (1925) who used vinegar as treatment. Embody (1924) reported examining trout (brook, brown and rainbow) at New Jersey Hatchery at Hackettstown. Gowanlock (1927) experimented on the control of *Gyrodactylus* in *Fundulus*. Guberlet *et al.*, (1927) reported *Gyrodactylus* attacking rainbow trout (which he ascribed to the subspecies *Salmo gairdneri shasta* Jordan) in a hatchery near Seattle, Washington. He stated that the disease at that time was widespread among the hatcheries of the Northwest. In spite of the fact it was common, hatchery attendants had never attempted to determine the percentage of infestation or the number of parasites per fish. Guberlet tried immersion baths with a variety of substances. Hess (1930) stated that most epidemics of trout occur among young fish that are about one inch in length. Laird and Embody (1931) worked with rainbows which had been parasitized by Discocotyle salmonis Schaffer, which was described for the first time on rainbow trout at Cold Spring Harbor, New York.

Other contributions to the knowledge of monogenetic trematodes were made by Atkins (1901) who reported Gyrodactylus as the agent of an epidemic which had been reported by the U. S. Commissioner of Fish and Fisheries for the year of 1899 at Craig Brook Station, Maine. Cooper (1915) reported the occurrence of G. medius Katariner from Canadian freshwater fishes. Van Cleave (1921) reported a new species of Gyrodactylus from Ameiurus melas. Johnston and Tiegs (1922) presented a reclassification of the superfamily Gyrodactyloidea. Mueller (1936a) presented a suggestion to separate the species Gyrodactylus elegans Nordmann, 1832 in two varieties A and B, the variety A being the one secured from goldfish and variety B the one recovered from trout. Price (1937) in his revision of the superfamily Gyrodactyloidea, also presented diagnosis and keys for the suborders of Monogenea, for the superfamilies of Monopisthocotylea, for the families of Gyrodactyloidea, for the subfamilies of Gyrodactylidae and for the genera of Gyrodactylinae.

Mueller (1937a) continued the studies of the North American Gyrodactyloidea, and stated that in his point of view the genus Gyrodactylus appeared to be usually on the body surface of the host, rarely on the gills. He cited G. elegans as a parasite of trout. Mizelle (1937) stated that serious damage to fish by ectoparasitic trematodes, especially under hatchery conditions, had been frequently reported. This economic aspect is probably in part responsible for the early control phase of the investigation in contrast to the later taxonomic studies. Mizelle in the same paper also alluded to other author's citations on the viviparity of Gyrodactylus as being conducive to the production of epidemics among fishes. It is interesting that even at that time Mizelle noticed that fish culturists and conservation agencies responsible for rearing and distributing fishes gave little attention to the dangers of transporting gill parasites into new regions. During the project this author observed that planting of fish took place exactly when the Gyrodactylus population was at its peak. Spronston (1946) in a comprehensive study of the monogenetic trematodes listed 25 species of *Gyrodactylus*, from which only *G. elegans* var. B as related by Mueller (1936a), was found to parasitize trout. Yin and Spronston (1948) gave the varieties A and B of *Gyrodactylus elegans*, as proposed by Mueller, subspecific status naming them *Gyrodactylus elegans muelleri* and *Gyrodactylus elegans salmonis*, respectively.

Turnbull (1956) listed 40 species of *Gyrodactylus* and made the first studies on the complete life cycle of a species of this genus.

Bychowsky (1957) contributed with another comprehensive paper on the systematics and phylogeny of the monogenetic trematodes. Ikezaki and Hoffman (1957) stated that it was believed that if the periods of gestation could be determined, the approximate density of parasites in a raceway could then be predicted. The authors did not cite the experiments done by Turnbull one year before. Malmberg (1962) presented an analysis of many problems of the genus *Gyrodactylus* and his method for the revision of the genus. Putz and Hoffman (1963) gave a synopsis of the genus *Gyrodactylus* found in North American fishes. In their list there were not any references on the genus *Gyrodactylus* for the State of Michigan. Rogers and Wellborn (1965) brought to 27 the number of valid species of *Gyrodactylus* plus the unnamed species of Hargis (1953 and 1955). Mizelle and Kritsky (1967b) described the third species of *Gyrodactylus* from *Salmo* gairdneri. They also provided a key for the North American species of this genus. The three species which were so far recorded from S. gairdneri are G. elegans salmonis (Nord.), G. colemanensis Mizelle and Kritsky, 1967 and G. brevis Crane and Mizelle, 1967. The same authors amended the generic diagnosis of the genus Gyrodactylus. One of the latest contributions to knowledge of the monogenetic trematodes was given by Unnitham (1971) where he wrote about the functional morphology of a new fauna of Monogenoidea of fishes.

For the digenetic trematode Crepidostomum, there are many references to cite, but among the more important is Brown (1927) with the life history of Crepidostomum farionis. Hunninen and Hunter (1933) described two more species of this genus. Hopkins (1931a and 1931b) contributed with the description of two more species, with a monograph on the papillose Allocreadiidae (Hopkins, 1934a) and describing C. brevivitellum (Hopkins, 1934b). In 1937, Ameel and Abernathy in separate papers contributed to the knowledge of the life history of C. farionis. Manter in 1970 (unpublished) recorded C. farionis from rainbow trout at Gretna State Fish Hatchery, near Lincoln, Nebraska.

On the acanthocephalans it is possible to cite among others the work by Van Cleave and Lynch (1950) on the circumpolar distribution of Neoechinorhynchus rutili. Kastak and Zitnan (1960) wrote about the age dynamics of N. rutili.

The life history of *N. rutili* was studied by Walkey (1962) and by Merritt and Pratt (1964). Walkey (1967) wrote about the ecology of *N. rutili*.

Echinorhynchus salmonis was recorded among others by Bangham (1955) from Lake Huron and Manitoulin Island, Ontario. DeGiusti and Budd (1959) have worked with the intermediate host of the helminth. Van Cleave (1920) contributed to the knowledge of this species and Ward (1937) wrote about variations in the female of this acanthocephalan from *Lota vulgaris*.

A seasonal distribution study of this acanthocephalan was made by Van Cleave in 1916.

MATERIALS AND METHODS

Collection of Fish

The fish from the hatchery were collected with a dip net. The fish from Wolf Lake and from Manistee River were collected with hook and line. The Department of Natural Resources of the State of Michigan provided the information that at Larry Gunia's Resort on the Manistee River I could be supplied with fish by the amateur fishermen. So all the rainbows were collected within a range of not more than three miles up and down stream from the resort which is located 18 miles upstream from Lake Michigan.

Collection and Preservation of the Parasites

The procedure for collection and preservation varied with the type of parasite and also, with the place of collection of the trout.

The raceway fish at the hatchery were sampled and brought back to the laboratory in an ice chest containing 20 liters of water taken from the raceway. No deaths occurred during transportation. The same procedure was followed for the Wolf Lake trout, but some times they would arrive in the laboratory in bad condition. I attributed this to the fact that they were caught with a hook which might have weakened the fish to some extent.

The trout from these two places, hatchery and lake, were killed one by one by piercing the heads. After that, the fins were cut off and put in Petri dishes containing water from the ice chest. The body always was examined for ectoparasites under a stereomicroscope giving magnifications from 14X to 60X. Following this, the head was cut off and the gills detached. The gill arches were examined individually. An incision was made, beginning at the isthmus and extending to the level of the cloaca. Care was taken to remove the whole digestive tract without rupture of any nature. At this time the body cavity was searched for coelomic helminths. Then, every organ was separated and put in individual Petri dishes of various sizes depending on the size of the organs. The following locations were searched: eyes, stomach, intestine, pyloric ceca, gall bladder, urinary bladder, and air bladder. I always recorded and preserved any food itens found in the stomach of the trout caught in the lake and in the river.

The organs were placed in 0.7% saline with the exception of the gills which were placed in water from the raceway.

All organs were slit open and carefully examined using the stereomicroscope. The stomach and the intestine were afterwards, put into a small jar to which was added 0.7% saline. The contents of the jar were shaken and then left to settle. Following this, the sediments were washed as well as the organs which were examined a last time. The material resulting from the washing was poured into a Petri dish and examined by parts depending on the amount. This part of the procedure was also followed for the rainbows from the Manistee River.

The parasites were killed in different ways depending upon their type.

Monogenetic trematodes when found were picked up one by one and deposited in watch glasses. They were killed with formalin 1:4,000. A modification of the method for mass collection of Gyrodactylus described by Parker and Haley (1960) was used but the results were unsatisfactory. Digenetic trematodes were first washed and then put between a slide and a cover slip in a Petri dish. Then A. F. A. (Alcohol-Formalin-Acetic Acid, formula as given by Cable, 1966) was added after little vials had been put over the sides of the cover slip with adjusted amounts of water to work as weights. This method of compression is very useful as it can be used for any size of trematode since it is only necessary to fill the vials until the desired weight is reached. The process must be watched under the stereo-microscope. The nematodes were killed by pouring hot A. F. A. over them after having them cleaned and stretched as much as possible in a Petri dish, without liquid. They were preserved in 70% alcohol-glycerine in a 50/50 proportion. The acanthocephalans were collected one by one with a very delicate forceps. Throughout the collection they were deposited in a container with saline. They were left in tap water in the refrigerator overnight and then fixed with hot A. F. A. Their preservation was done with 70% alcohol.

Preparation of the Parasites for Study

The monogenetic trematodes were transfered to a solution of 70% alcohol-glycerine and from there they were transfered to pure glycerine. Mounting was in glycerine jelly.

The digenetic trematodes were treated with the following sequence after fixation in A. F. A.:

30% alcohol - 15 min.

50% alcohol - 15 min.

Langeron's Carmine - 30 min.

0.5% hydrochloric acid in 70% alcohol - until good differentiation.

30% alcohol - 15 min.

50% alcohol - 15 min.

70% alcohol - 15 min.

90% alcohol - 15 min.

absolute alcohol - 15 min.

xylene - until clarification

creosote - at least one day

Mounting was in Permount.

The nematodes were retained for later study.

The acanthocephalans were treated with the same sequence as the digenetic trematodes, only that the time intervals were longer.

RESULTS

Descriptions of the Parasites

In this project four genera of helminths were identified: one monogenetic trematode, one digenetic trematode, and two acanthocephalans. The generic diagnosis given here is the latest available for the given genus.

Trematodes

Phylum Platyhelminthes
Class Trematoda
Order Monogenea Carus, 1863
Suborder Monopisthocotylea Odhner, 1912
Superfamily Gyrodactyloidea Johnston and Tiegs, 1922
Family Gyrodactylidae Cobbold, 1877
Genus *Gyrodactylus* Nord.,1832 amend.
(Fig. 1, B)

Generic diagnosis as given by Mizelle and Kritsky (1967a)

> Gyrodactylidae: body elongate, divisible into a cephalic region, trunk, peduncle and haptor. Cephalic area usually cleft producing two terminal cephalic lobes usually containing one or more spicules and portions or all of the head organs. Eyes absent. Pharynx compound, composed of two

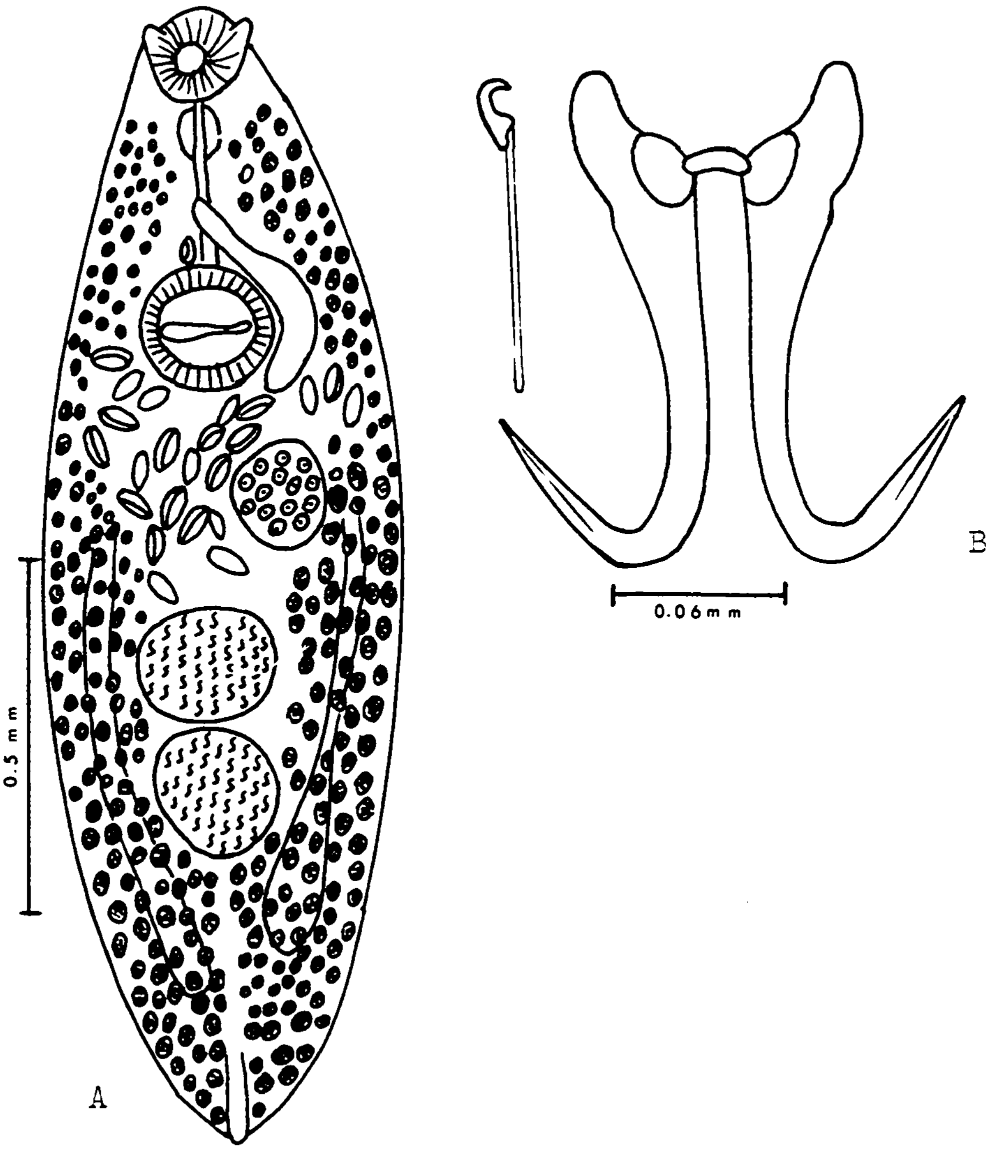


Fig. 1. Trematodes. A. Crepidostomum farionis . B. Anchors, deep bar, and hooklet of Gyrodactylus sp.

hemispherical or subhemispherical parts; esophagus short, intestinal crura (two) usually without diverticula and terminating blindly in posterior part of trunk or in peduncle. Testis median, postuterine, inter or postcecal. Cirrus ventral, median or submedian situated below or immediately posterior to level of pharynx, and armed with one spine and one to several spinelets. Genital pore ventral, submedian, and postpharyngeal. Ovary usually posttesticular and median. Uterus central or subcentral, usually containing an embryo which may contain an embryo of the succeeding generation. Vitellaria composed of individual masses at level of, or posterior to termination of crura. Vagina and genito-intestinal canal absent. Haptor ventrally concave possessing one pair of anchors (ventral) with bases connected by a superficial and a deep bar; and 16 radially arranged hooks of similar shape and size (usually) whose points project to produce a scalloped haptoral border. Parasites of external surfaces and gills of freshwater and marine fishes.

<u>Gyrodactylus</u> sp.

Location: external surface

Host: <u>Salmo</u> <u>gairdneri</u> Rich.

Locality: Wolf Lake State Fish Hatchery

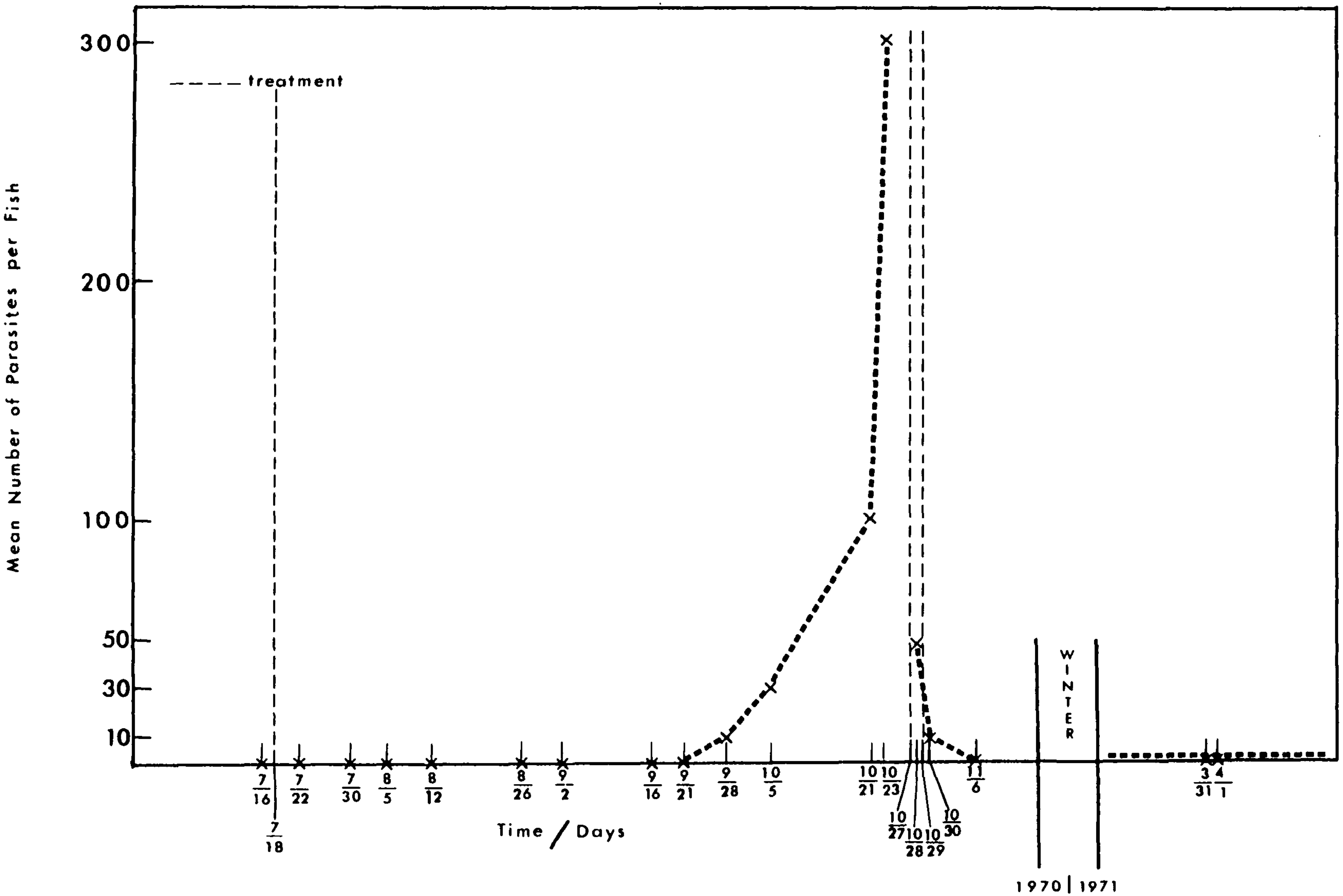
<u>Gyrodactylus</u> sp. was recorded only from the hatchery environment. The parasites were absent in the earliest samples taken, as can be seen in Table 1. The parasites developed a population, which when control measures were taken, had already reached more than 300 individuals per fish examined. The progress of the population growth can be seen in Fig. 2.

Table 1. Chronological list of rainbows examined, age, size, size of the samples and estimated number of Gyrodactylus sp. / fish.

Sampling date	Average size in cm.	Age in months	Formalin treatment	Size of samples	Estimated number of Gyrodactylus sp. / fish *
7/16/70	13	9	-	3	0
7/22/70	15	9	-	9	0
7/30/70	15	9	-	4	0
8/05/70	16	9	-	2	0
8/12/70	16	9	-	4	0
8/26/70	16	10	-	5	0
9/02/70	17	10	-	5	0
9/16/70	17	11	-	4	0
9/21/70	18	11	-	5	0
9/28/70	18	11	-	3	10
10/05/70	18	11	-	5	30
10/21/70	19	12	-	3	100
10/23/70	21	12	-	3	300
10/27/70	-	-	1:4,500	-	-
10/28/70	21	12	-	5	50
10/29/70	-	-	1:6,000	-	-
10/30/70	23	12	-	5	10
11/06/70	23	12	-	5	0
3/31/71	10	5	-	25	0.2
4/01/71	10	5	-	24	0.3

* This estimated number is based on a count of all Gyrodactylus seen. Loss of specimens prior to counting and some missed individuals probably make this number a minimum figure.

Fig. 2. Increase in numbers of the Gyrodactylus population.



Order Digenea Carus, 1863

Superfamily Allocreadioidea Nicoll, 1934

Family Allocreadiidae Stossich, 1903

Genus *Crepidostomum* Braun, 1900

(Fig. 1, A)

Generic diagnosis as given by Hoffman (1970)

> Allocreadiidae: body elongated-oval to subcylindrical. Oral sucker terminal, surmounted anterodorsally by a half-crown of two to six head papillae. Mouth aperture ventroterminal or ventral. Prepharynx present, pharynx well developed. Esophagus short or moderate. Ceca terminate near posterior extremity. Ventral sucker in anterior half of body. Testis tandem (sometimes appear as four), in posterior half of the body. Cirrus pouch more or less elongated claviform: overreaches ventral sucker; contains seminal vesicle, prostatic complex and ductus ejaculatorius. Genital pore median, pre-acetabular. Ovary submedian, between ventral sucker and anterior testis. Seminal receptacles and Laurer's canal present. Uterus coiled between anterior testis and ventral sucker. Vitellaria circumcecal, extending from forebody to posterior extremity. Excretory bladder reaches to or beyond anterior testis. Life cycle: adult in fish; oculate xiphidiocercaria in sphaerid clams; metacercaria in aquatic insects, usually mayflies, and crustacea. With 9 species.

Crepidostomum farionis (Müller, 1784)

Location: intestine and gall bladder

Host: *Salmo gairdneri* Rich.

Locality: Wolf Lake

Seventy specimens were recovered from the intestine and three from the gall bladder. All the specimens from the intestine were mature and the three specimens from the gall bladder were juvenile. These two groups of *C. farionis* were found in the same host from Wolf Lake. They were the only digenetic trematodes recovered throughout the project. The mature specimens have a typically tapered body at both ends and contrast with the two groups of trematodes of the same species which were collected also from *S. gairdneri* at Gretna State Fish Hatchery, near Lincoln, Nebraska. The trematodes from Nebraska are much shorter and wider and also present differences in the sucker ratio as well as in the length of the cirrus pouch.

Acanthocephalans

Phylum Acanthocephala

Order Neoechinorhynchidea Southwell and Macfie, 1925

Family Neoechinorhynchidae Van Cleave, 1919

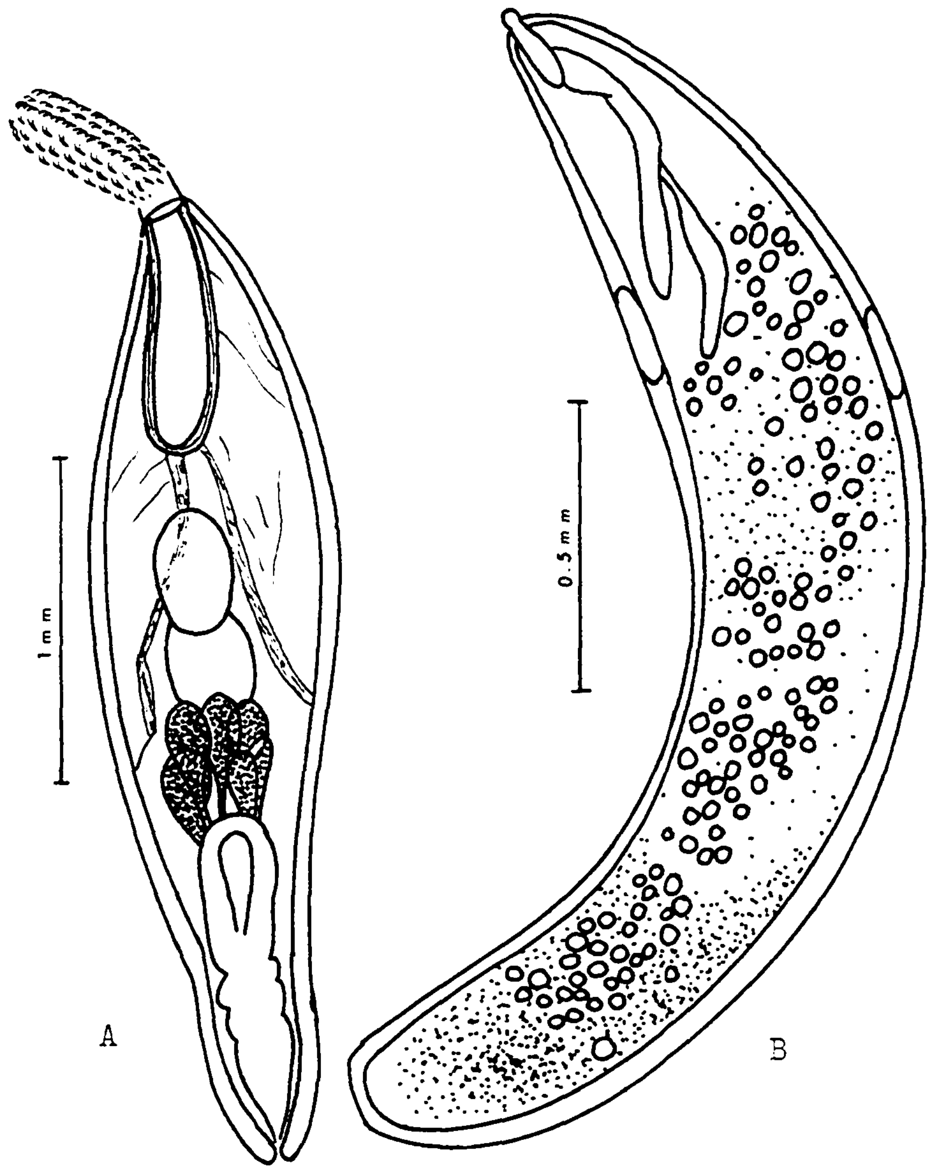
Genus *Neoechinorhynchus* Hamann, 1892

(Fig. 3, B)

Generic diagnosis as given by Hoffman (1970)

> Neoechinorhynchidae: body usually small, cylindrical, bowed or stright. Lacunar system with median (dorsal and ventral) longitudinal vessels and circular vessels with anastomoses. Giant hypodermal nuclei almost always few (usually 4 or 5 dorsally and 1 or 2 ventrally). Proboscis short, somewhat globular;

Fig. 3. Acanthocephalans. A. *Echinorhynchus salmonis*, male. B. *Neoechinorhynchus rutili*, female.



proboscis hooks in 6 spiral rows of 3 each (usually referred to as 3 circles of hooks) anterior hooks longer and stouter than others. Proboscis receptacle subcylindrical, rather short, single layered with ganglion at or close to its base. Lemnisci, digitiform to filiform, long, with few giant nuclei. Testes contiguous, or not, at or near midregion, sometimes in posterior half of trunk. Cement gland syncytial with several nuclei; cement reservoir rounded overlapped by cement gland. Eggs oval to elliptical, with concentric shells. Life cycle: adult in marine and freshwater fish, frogs and turtles; larva in small crustacea; some species also have a second intermediate host; larval form very similar to adult.

Neoechinorhynchus rutili (Müller, 1776)
(Syn.: *Echinorhynchus tuberosos* Zider, 1803)

Location: intestine

Host: *Salmo gairdneri* Rich.

Locality: Wolf Lake

Only one mature female was found..It was the only representative of this species found throughout the survey. It is possible that with a larger sample of Wolf Lake trout a larger incidence as well as a larger intensity of infestation would be found.

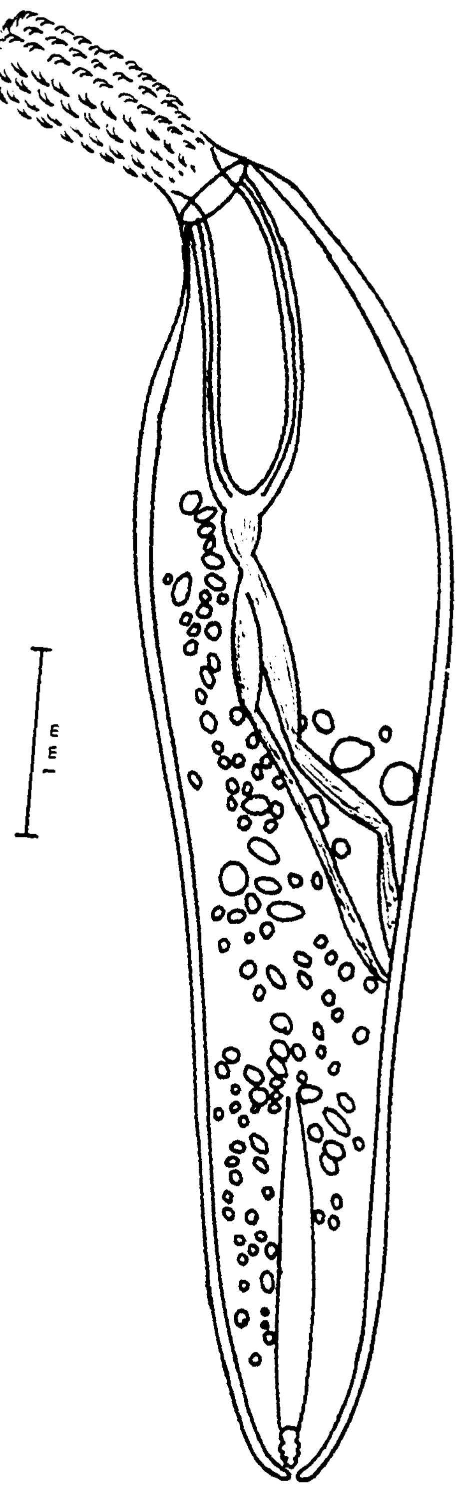
Order Echinorhynchidea Southwell and Macfie, 1925

Family Echinorhynchidae (Cobbold, 1879)

Genus *Echinorhynchus* Zoega *in* Müller, 1776

(Fig. 3, A and 4)

Fig. 4. Echinorhynchus salmonis, female.



Generic diagnosis as presented by Hoffman (1970)

> Echinorhynchidae: body small to medium sized, hypodermal nuclei small, numerous. Lacunar system with lateral main vessels and reticular anastomoses. Proboscis long, cylindrical, directed ventral, with 9 to 26 longitudinal rows of 5 to 16 hooks each; root of hook simple, becoming smaller toward the base of proboscis, where it disappears. Proboscis receptacle cylindrical to claviform, double walled, with ganglion near middle. Lemnisci usually claviform. Testes oval to elliptical, tandem, contiguous or not, in middle third of trunk. Cement glands 6, more or less compact, one behind another or close together. Eggs much elongated, fusiform with prominent polar prolongations of middle shell. In freshwater and marine fishes. Life cycle: larva in amphipods; no second intermediate host.

Echinorhynchus salmonis Müller, 1784 (Syn.: *E. coregoni*, *E. pachysomus*, *E. phoenix* *E. inflatus*, *E. maraense*, *E. murense*)

Location: intestine

Host: *Salmo gairdneri* Rich.

Locality: Manistee River

E. salmonis was found with a 100% incidence in the rainbows from the Manistee River. There were from 3 to 173 specimens per host fish. The fish probably became infested in Lake Michigan before the migration upstream.

The Hatchery Environment

Wolf Lake State Fish Hatchery is located on highway M-43, 6 miles west of Kalamazoo, in sections 13 and 14 of Almena Township, Van Buren County, Michigan. The hatchery has 21 ponds in operation, two buildings where 21 tanks are located and 4 raceways (Fig. 5). There are no special purpose tanks or ponds and treatment other than for fungus and bacteria was not being done at the time of the study.

The source of water for the tanks and raceways is a spring located by the highway M-43. The water is pumped to the main building where the young fingerlings are reared, and passes in parallel through the 11 tanks. Then the water is recollected and sent to the egg house, where it passes through 10 more tanks and the egg battery. From this second building the water flows to a show pond in which several species of fish, including trout, are maintained for visitor's observation. There are two outlets in the show pond, discharging some 20 meters apart from each other, into a small creek across the road. Apparently there are a few trout in this small creek. Examination of gastropods of the genus Physa, during the sampling time disclosed no larval trematodes. The water from the creek then flows into the raceways and from there it goes to Wolf Lake.

The fish examined from the were all from a population

Fig. 5. Aerial photograph of Wolf Lake State Fish Hatchery and Wolf Lake.

kept in the raceways. They were placed in the 0+ age group. The age groups were established with a chart distributed by the Michigan Department of Natural Resources (1969). A random sample of 2 to 9 fish was collected from the middle of the school at each time. The collection of fish which were at the surface, or swimming out of the school, was always avoided. On the 31st of March of 1971 and 1st of April of the same year a follow up study was made in which the number of fish in the sample increased to 25 and 24 respectively. The population in the raceway was constituted of approximately 23,000 trout, until October 26 when 20,000 rainbows were removed. The 1971 samples came from a diffeerent population placed in the same raceway.

The Lake Environment

Wolf Lake is located just north of the raceways of the Wolf Lake State Fish Hatchery (Fig. 5) in section 13 of Almena Township. It is a small lake of about 12 hectares, has 3 inlets and 1 outlet.

The samples were taken at the level of the raceways outlet. Nineteen trout were throughly examined. The rainbows measured from 15.5 cm to 34 cm in length and were placed in the I+, II+, III+ and IV+ age groups, (Table 2).

No monogenetic trematodes were found nor were parasitic

Table 2. Chronological list of fish examined from Wolf Lake, age group, and parasites recovered.

Exam #	Sampling date	Size in cm	Age group	Parasites				
				Mon. Trem.	Dig. Trem.	Cest.	Nem.	Acant.
26	7/31	23	III+	-	-	-	-	-
27	7/31	30	IV+	-	73	-	-	-
28	7/31	25	III+	-	-	-	-	-
29	7/31	23	III+	-	-	-	-	-
30	7/31	18	II+	-	-	-	1	-
33	8/7	20	II+	-	-	-	-	-
34	8/7	17	II+	-	-	-	-	-
35	8/7	16	II+	-	-	-	16	-
36	8/7	15.5	I+	-	-	-	-	1
37	8/7	17.5	II+	-	-	-	-	-
42	8/13	30	IV+	-	-	-	-	-
43	8/13	31	IV+	-	-	-	-	-
44	8/13	24	III+	-	-	-	-	-
45	8/13	33	IV+	-	-	-	-	-
46	8/13	34	IV+	-	-	-	-	-
57	9/4	30	IV+	-	-	-	-	-
58	9/4	25	III+	-	-	-	-	-
60	9/4	20	II+	-	-	-	-	-
61	9/4	18	II+	-	-	-	-	-

crustaceans or annelids present. The only digenetic trematode found was Crepidostomum farionis (Fig. 1, A). All the trematodes were recovered from a single host. Three rainbows contained nematodes in the stomach. The only acanthocephalan recorded was recovered from the smallest trout. It was the only representative of the species Neoechinorhynchus rutili (Fig. 3, B).

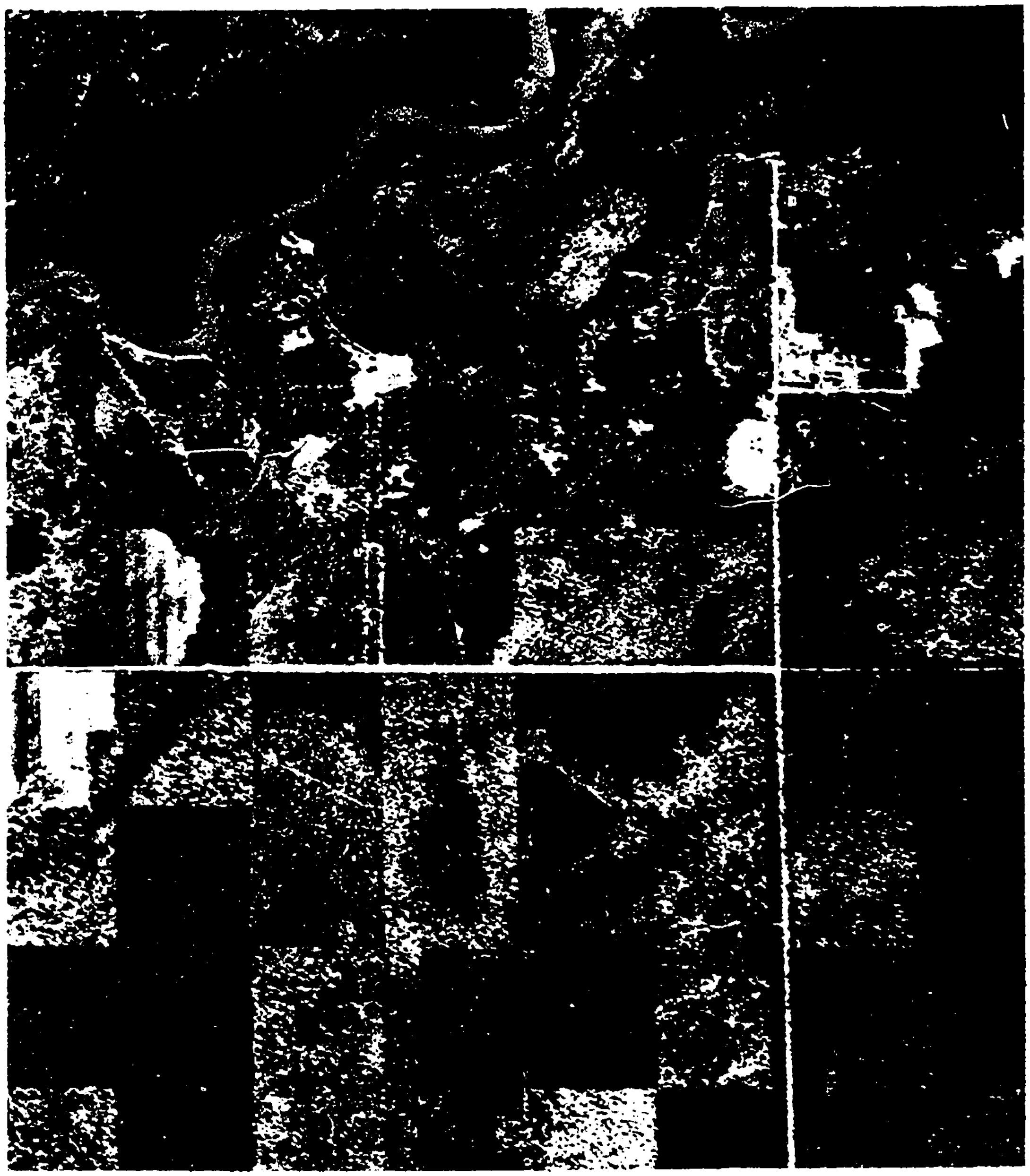
The River Environment

The Manistee River is located in the northwest part of the lower peninsula of the State of Michigan. It rises in lakes in southwest Otsego County and flows generally southwest. It is about 170 miles long and flows past Mesick and through the Manistee National Forest to Lake Michigan, west of Manistee. It widens into Manistee Lake just east of Manistee, before it enters Lake Michigan.

The station was established at Larry Gunia's Resort, 13 miles upstream. The laboratory was set up in a hut used as a shop for tackle and the fish were examined as they were brought by sport fishermen.

The rainbows were taken from the portion of the river which appears in the map (Fig. 6). A total of 22 rainbow trout which were caught in their upstream migration from Lake Michigan were examined. External parasites were never considered due to the fact that the trout were always brought with

Fig. 6. Aerial photograph of the collection site on the Manistee River at Larry Gunia's Resort.



the external surface dry. Table 3 shows the period in which the examinations were made, as well as the number of *E. salmonis* recovered from each fish host. The age group of the fish and their size; the incidence, the average number and the total number of *E. salmonis* are also indicated. Table 4 shows the individual number of *E. salmonis* as well as the total number of that acanthocephalan per age group and per fish host. The data from Table 3 are graphically shown in Fig. 7.

Table 3. Chronological list of fish examined from the Manistee River, their size, age group and number of *E. salmonis* per fish.

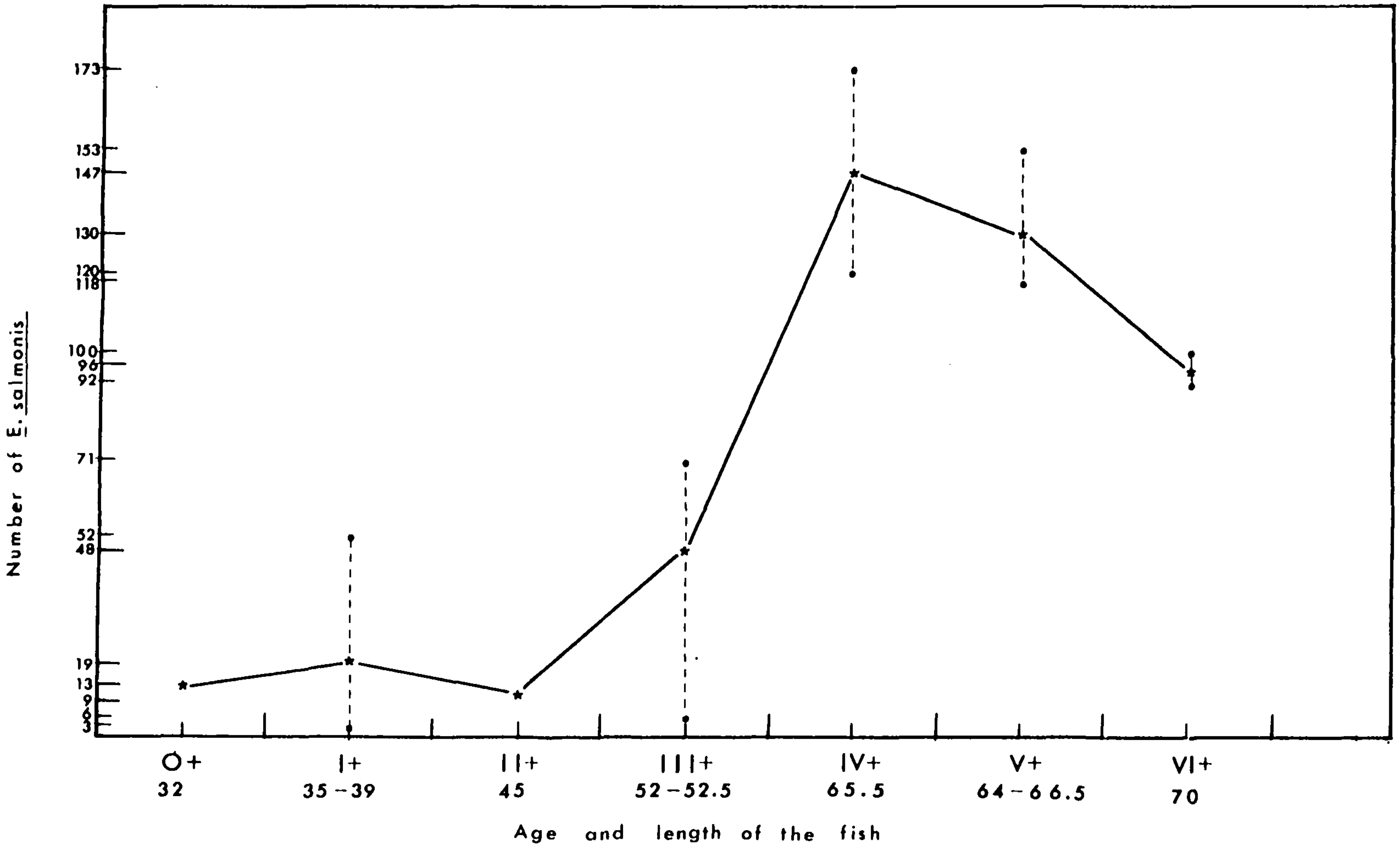
Exam #	Sampling date	Size in cm	Age group	Number of E. salmonis
71	9/22	52.5	III+	6
72	9/23	39	I+	3
73	9/24	35	I+	8
77	9/30	66.5	V+	118
78	10/1	62.5	IV+	173
79	10/1	37	I+	20
80	10/1	37	I+	3
81	10/1	62.5	IV+	120
82	10/1	52	III+	43
86	10/14	70	VI+	92
87	10/14	70	VI+	100
88	10/14	53	III+	71
89	10/15	64	V+	111
90	10/15	52.5	III+	71
91	10/15	64	V+	120
92	10/15	37.5	I+	130
93	10/15	36	I+	52
94	10/16	45	II+	9
95	10/16	64	V+	153
96	10/16	32	0+	13
97	10/16	36	I+	11
98	10/16	36	I+	5

Incidence of *E. salmonis*: 100%
Average number of *E. salmonis* per fish: 65.09
Total number of helminths revovered: 1,432

Table 4. Number of E. salmonis per fish per age group

Age group	Number of E. salmonis per fish						Total number of E. salmonis per age group	Average number of E. salmonis per fish
O+	13	-	-	-	-	-	13	13
I+	3	8	20	3	52	30	116	19
II+	9	-	-	-	-	-	9	9
III+	6	43	71	71	-	-	191	48
IV+	120	173	-	-	-	-	293	147
V+	118	120	153	-	-	-	391	130
IV+	92	100	-	-	-	-	192	96

Fig. 7. Relationship between the age and length of rainbow trout from the Manistee River and the intensity of infestation by E. *salmonis*. (Roman numerals represent the age group of the trout).



The Food in the Three Environments

During the study, the stomachs were always examined with great care and all food found was recorded and organisms were preserved for later study.

The rainbows from the hatchery had only fish meal in the stomachs. The rainbows from Wolf Lake on the other hand presented a variety of organisms in some instances, and in others had no food at all. The organisms most commonly found were chironomid larvae, the isopod, *Asellus* sp., and the snails, *Physa* sp., *Gyraulus* sp. and *Amnicola* sp. While the mollusks were found in very few instances, the crustaceans and the midges were present in almost all rainbows. Most of the time the midges were found in larger numbers.

In the Manistee River trout the stomachs were found to contain little or no food. When there was something it was spawn bags (used as bait), corn kernels, beetle larvae, gryllids, coleopterans and in one instance, viscera of fish.

Pathology

The raceway trout always had fungi on the fins. Sometimes the fins were merely small stubs. When monogenetic trematodes were found, fungi were present in greater amounts.

The rainbows from the lake did not exhibit any sign of disease either externally or internally.

The Manistee River rainbows, on many occasions, had intense hemorrhage along with the acanthocephalans in the intestine. It was possible to see that each helminth had the proboscis completely buried in the intestinal wall which had a circular edema around the wound. There were instances when the proboscis went through the intestinal wall.

DICUSSION

The environmental parameters which were present in the raceway can be considered almost constant. For instance, there were 23,000 rainbow trout living in the raceway which had a flow rate of 190 to 220 liters of water/ min./ raceway and a narrow temperature range varying only from 11 to 13 degrees Centigrade. The absence of large invertebrate organisms plus the fish meal diet, contributed to eliminate the possibility of infestation of the trout by helminths with indirect life cycles. On the raceway trout the only helminth parasite species found was one which reproduces directly, with favorable, stable environmental parameters and a fish crowding situation. There have been others hatcheries infested with helminths which have an indirect life cycle, but in those, fish-eating birds and invertebrates were allowed in the raceway environment which was not the the case at Wolf Lake State Fish Hatchery.

The population of Gyrodactylus sp. increased in numbers during a long interval between treatments with formalin. The concentration of 1:6,000 and even 1:4,500 which were applied with the purpose of killing fungi and bacteria, may not have killed the remnant parasites of the previous populations of fish which always were transferred from the hatchery. In spite of the daily cleaning process of the raceways, there may have been times between transfer of fish when helminths were left long enough to attach themselves to the next population of rainbows. They may also have been imported from the show pond or small section of stream above the raceway. The low species diversity in the raceway may be credited to the lack of intermediate hosts. The high intensity of infestation by the monogenetic trematodes may be credited to the non-specific concentration and non-periodical treatments with formalin. Fig. 2 shows graphically how the *Gyrodactylus* population increased from zero to more than 300 helminths per fish examined. The very high rise in the parasite number from October 21 to 23 may be explained in terms of the gestation period of the embryos which is about 18 hours from birth of an embryo to the birth of the succeeding one, if the species under consideration here has a life cycle like *G. bullatarudis* Turnbull, 1956.

Population growth rates during the period from detection of *Gyrodactylus* and the beginning of control are as follows:

Sept. 28 - Oct. 5	2.8 flukes/day
Oct. 5 - Oct. 21	4.4 flukes/day
Oct. 21 - Oct. 23	100 flukes/day

For the whole period from Sept. 28 to Oct. 23, the population growth rate was 11.6 flukes/day. If we assume that the flukes were expanding exponentially in a favorable environment such that

$$N_t = N_o e^{rt}$$

where

N_t is number at time t

N_o , number at time o

e, the base of natural logarithm

and r, the intrinsic rate of natural increase (Andrewartha and Birch, 1954).

The values of r for the three periods of time are:

Sept. 23 - Oct. 5		r = 0.156/day
Oct. 5	- Oct. 21	r = 0.069/day
Oct. 21	- Oct. 23	r = 0.549/day

Also, r may be calculated for the whole period of time, to have a value of 0.136/day.

Accepting the data at face value, r was, however, not a constant but was highest between Oct. 21 and 23 (r = 0.549) and lowest between Oct. 5 and 21 (r = 0.069). Whether these differences are based on life history or environmental factors or whether they are merely a product of sample variability owing to inadequate size of the sample, is unknown.

The Wolf Lake population sample exhibited a very low incidence and intensity of parasitism, however, these parasites showed the largest taxonomic variation of the three environments sampled. Data concerning the fish and the parasites found in the lake are shown in Table 2. The rainbow population in Wolf Lake is all planted and sometimes it is enlarged by the fish which escape from the raceways of the hatchery. On the other hand those fish presented a food composition in their stomachs (crustaceans and mollusks) which could work as intermediate hosts for helminths. So the low incidence and low intensity of parasitism may be credited to the fact that the rainbows in the lake comprised a recent introduction. Also, the low range in age of the fish caught might have been a reason as well as the lower population level. If older fish had also been caught maybe the number of parasite species as well as the incidence and intensity would have been larger. Even the small number of species found shows that there are all the necessary types of intermediate hosts at Wolf Lake to secure an infestation with at least trematodes, nematodes and acanthocephalans.

The Manistee River rainbows, are in a different situation in relation to the rainbows from the other two environments. They are not river residents. Those fish live most of the year in Lake Michigan and during the spawning season go upstream carrying with them parasites which are the product of Lake Michigan's intermediate hosts infestation. In the rainbows from the river a low species diversification was found, as only Echinorhynchus salmonis was found. Another fact that could account for the low species diversification is that many of them were planted fish, although the maximum incidence of 100% in which E. salmonis was found seems to weaken that fact. Also, the relatively high intensity (sometimes more than 100 helminths per fish) and the mean intensity of 65.09 parasites per fish may indicate that when the fish live near a population of intermediate hosts which are infested with viable larval forms of helminths, they do become infested. I have said relatively high intensity because when it is compared with data of Hoffmann (1954), it is not so large. That author examined Salmo trutta L. and very often found intensities of over 150 Echinorhynchus truttae, and in one instance recorded 1,700 acanthocephalans in the intestine of a brown trout.

The fact that I never found any amphipods in the stomach of the 22 trout examined suggests that the fish did not become infested when in the river and that presumably the vector amphipods for E. salmonis are in Lake Michigan.

It is possible that most of the trout caught 18 miles upstream in the Manistee River had come from relatively small area in Lake Michigan. A survey of the plankton and bottom fauna around the mouth of the Manistee River, would bring more light to the problem.

As I found the population of E. salmonis formed by helminths of all sizes, I am inclined to state that the infestation by this parasite occurs at all times in the lake when feeding on the crustacean population. They do not show a seasonal regulation, according to my data.

The only food found in the stomachs of the river rainbows was many types of arthropods, salmonid eggs, etc. This is in accordance with the data recorded by Shapovalov and Taft (1954) and Hildebrand (1955) where the later listed many tables in which the main groups of organisms recorded were arthropods.

Dogiel _et al._, (1958) stated that parasitological data can be used as indicators of the diet of the hosts, and added that to be useful as indicators the parasites must be reasonably common in the fish, infesting at least 25% of the examined population. Thus, amphipods would seem to be a very significant food item of rainbows in Lake Michigan. Another possibility which may be considered is that the trout are feeding upon smaller fishes which in turn feed on the amphipods, the smaller fishes thus acting as transport hosts for the larval acanthocephalans.

Fish are very well suited to the study of the influence with the host's age has on its parasite fauna (Dogiel _et al._, 1958). The same authors stated that in the case of the fish _Esox lucius_ it was possible to assemble the whole parasitological fauna in different groups in relation to the age of the fish host and to the number of parasites: a. Parasites indepedent of the age of the host; b. Parasites decreasing in abundance with age of the host; c. Parasites increasing with age of the host. The authors added that the third group is undoubtedly predominant. I believe

E. salmonis would belong in the third group if such a system would prove valid for the parasites of *S. gairdneri* in this region. The data in which the age of the hosts is plotted against the number of parasites per fish is shown in Fig. 7.

Another good indicator of the increase in parasitization with the age of the fish host is the average number of parasite species present in hosts of various ages. Petrushevski (1937) found that the number of parasite species in cultured trout in U. S. S. R., increased with age. His results showed that the 0+ age group trout were mostly free of parasites. The figures increased for the age groups I+, II+, and III+ respectively.

In this study, as can be seen in Fig. 7, the general trend was that *E. salmonis* also increased in intensity with the age of the host. Although the age groups 0+ and II+ had only one fish in each, they could fit the overall view that the intensity increases until the age group IV+ and then slightly decreases for the fish in the age groups V+ and VI+.

The number of species found was low. Larger samples might disclose a larger species diversity. Also a survey during all seasons of the year would probably add species of parasites not known from *S. gairdneri* in the Manistee River. Appendix I shows a list of helminth parasites of rainbow trout in North America.

Dogiel et al.,(1953) presented interesting data related to the seasonal fluctuations of the parasite fauna of a salmonid fish. They cited the work of Shulman and Shulman-Albova in which *E. salmonis* when recovered from *Coregonus lavaretus baeri* yielded a stable percentage of parasitism of 100% from August to November and a high mean intensity of parasitism. In spite of the fact that the hosts do not belong to the same genus, Shulman and Shulman-Albova's data and mine agree, with the exception that parasitism in the Manistee rainbows showed a higher mean intensity.

SUMMARY

1. The helminthological fauna of <u>Salmo gairdneri</u> Rich. was surveyed when this species of salmonid was secured from three different types of environments: a hatchery, a lake and a river.

2. The rainbows from the hatchery presented only <u>Gyrodactylus</u> sp. which during a six week interval built up a large population where more than 300 helminths could be found per fish.

3. The rainbows from the lake presented a varied helminthological fauna, although the incidence and the intensity of infestation were low.

4. The rainbows from the river presented a very low species diverification, but there was a 100% infestation caused by the acanthocephalan <u>Echinorhynchus salmonis</u>, which totaled 1,432 helminths recovered from 22 trout.

5. <u>E. salmonis</u> appears to belong to a group of helminths of <u>S. gairdneri</u> in the Manistee River, which increases in number with the age of the host.

6. Helminths dangerous to man were not found infesting rainbow trout in any of the environments.

RESUMO

1. A fauna helmintologica da truta arco-iris, Salmo gairdneri Rich. foi objeto de um estudo comparativo, quando coletada em um posto de piscicultura, em um lago e em um rio.

2. As trutas do posto de piscicultura somente apresentaram o trematodeo monogenetico Gyrodactylus sp., o qual num periodo de seis semanas desenvolveu uma populacao onde mais de 300 helmintos por peixe puderam ser encontrados.

3. As trutas provenientes do lago apresentaram a fauna helmintologica mais variada, embora a incidencia e a intensidade de infestacao registradas foram baixas.

4. As trutas provenientes do rio, apresentaram uma composicao especifica muito reduzida, apesar de que 100% estavam infestadas pelo acantocefalo E. salmonis. De 22 trutas examinadas foram recolhidos 1.432 helmintos.

5. E. salmonis, parece pertencer ao grupo de helmintos parasitos de S. gairdneri, que no Rio Manistee aumenta em numero proporcionalmente a idade do hospedeiro.

6. Nao foram encontrados parasitos a saude humana.

APPENDIX I

List of Helminth Parasites of *S. gairdneri* Rich. recorded in North America

Trematodes

Aponurus sp.	Shaw (1947)	Ore.
Crepidostomum cooperi Hopkins, 1931	Hopkins (1931)	Ontario
Crepidostomum farionis (Müller, 1784)	Haderlie (1953)	Calif.
Crepidostomum laureatum Cooper, 1915	Shaw (1947)	Ore.
Deropegus aspina (Ingles) McCauley and Pratt, 1961	McCauley and Pratt (1961)	Ore.
Diplostomulum of *Bulbophorus confusus* (Kraus, 1914)	Fox (1965)	Mont.
Diplostomulum of *Diplostomum spathaceum* (Rudolphi, 1819) Braun, 1893	Ferguson (1943)	N. Jersey
Diplostomulum of *Diplostomum spathaceum* (Rudolphi, 1819) Braun, 1893	Ferguson and Hayford (1941)	N. Jersey
Discocotyle salmonis Schaffer, 1916	Schaffer (1916)	N. York
Discocotyle sagittata (Leuckart, 1842) Diesing, 1850	Price (1943)	U. S. A.
Gyrodactylus brevis Crane and Mizelle, 1967	Crane and Mizelle (1967)	Calif.

Gyrodactylus colemanensis Mizelle and Kritsky, 1967	Mizelle and Kritsky (1967b)	Calif.
Gyrodactylus elegans Nord., 1832	Curtin (1956)	Calif.
Gyrodactylus elegans Nord., 1832	Haderlie (1953)	Calif.
Gyrodactylus elegans salmonis (*G. elegans* B Müller, 1936)	Yin and Spronston (1948)	N. Amer.
Nanophyetus salmincola Chapin, 1926	---	Ore.
Neascus sp.	Haderlie (1953)	Calif.

Cestodes

Cyathocephalus truncatus (Pallus, 1871)	Alexander (1960)	Ore.
Diphyllobothrium cordiceps (Leidy, 1871)	Vik (1964)	Ore.
Diphyllobothrium sp.	Fox (1962)	Mont.
Eubothrium crassum (Bloch, 1779)	Bangham (1955)	L. Huron
Eubothrium salvelini (Schrank, 1790)	Bangham and Adams (1954)	B. Col.
Eubothrium sp.	Griffith (1953)	Wash.
Proteocephalus pinguis LaRue, 1911	Bangham (1955)	L. Huron
Proteocephalus salmonidicola Alexander, 1951	Alexander (1951)	Ore.

Proteocephalus salmonidicola Alexander, 1951	Bangham and Adams (1954)	B. Col.
Proteocephalus salmonidicola Alexander, 1951	Haderlie (1953)	Calif.
Proteocephalus salmonidicola Alexander, 1951	Jones and Hammond (1960)	Utah
Proteocephalus tumidicollis Wagner, 1953	Wagner (1953)	Calif.
Proteocephalus tumidicollis Wagner, 1953	Wagner (1954)	Calif.

Nematodes

Bulbodacnitis globosa (Zeder, 1800) Dujardin, 1845	Bangham and Adams (1954)	B. Col.
Bulbodacnitis occidentalis Smedley, 1933	Haderlie (1953)	Calif.
Contracaecum sp.	Haderlie (1953)	Calif.
Cucullanus truttae (Fabricius, 1794)	Shaw (1947)	Ore.
Metabronema salvelini (Fujita, 1922)	Bangham (1955)	L. Huron
Metabronema salvelini (Fujita, 1922)	Haderlie (1953)	Calif.
Philonema agubernaculum Simon and Simon, 1936	Simon and Simon (1936)	Wyo.
Philonema oncorhynchi Kuitunen-Ekbaum, 1933	Haderlie (1953)	Calif.
Philonema oncorhynchi Kuitunen-Ekbaum, 1933	Shaw (1947)	Ore.
Philometra sp.	Shaw (1947)	Ore.

Acanthocephalans

Acanthcoephalus jacksoni Bullock, 1962	Bullock (1962)	Me.
Echinorhynchus leidyi Van Cleave, 1924	Bangham (1955)	L. Huron
Echinorhynchus salmonis Müller, 1784	Bangham (1955)	L. Huron
Leptorhynchus thecathum (Linton, 1891) Kostylew, 1924	---	---
Neoechinorhynchus rutili (Müller, 1766)	Bangham (1955)	L. Huron
Pomporhynchus bulbocolli (Linkins, 1919) Van Cleave, 1919	---	Ore.
Rhadinorhynchus sp.	Shaw (1947)	Ore.
Tetrahynchus sp.	Shaw (1947)	Ore.

Note: Complete citation for sources in this list, if not contained in Hoffman (1970), Dogiel *et al*., (1958), and Hargis *et al*., (1969).

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