STUDIES ON SOME INTERNAL HELMINTHS PARASITIC IN THE TROUT <u>SALVELINUS</u> FONTINALIS

(MITCHILL) IN QUEBEC

by

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A Thesis

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INTRODUCTION

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The speckled trout, Salvelinus fontinalis (Mitchill), is highly valued in the Province of Quebec as a game fish. However, little is known regarding the distribution and importance of the helminth parasites of this fish in Quebec, or the biology, the systematic status and morphology of some of the species involved. Accordingly, the present study was undertaken with the following objectives: (1) to describe the life history of the fluke Phyllodistomum lachancei and of the roundworm <u>Metabronema</u> <u>salvelini;</u> (2) to obtain additional data on the life history of the trematode Crepidostomum cooperi; (3) to determine the definitive host of a cestode, the plerocercoid stage of which occurs in the trout; (4) to describe the adult and larval stages of the trematode Phyllodistomum lachancei, the larval stages of the nematode Metabronema salvelini and the larva of an undetermined species of Spirurid; (5) to review the systematic status of species of the genera Crepidostomum, Proteocephalus, Rhabdochona and Metabronema parasitic in trout; (6) and to discuss the distribution and incidence of the fluke Crepidostomum farionis and of the cestodes Proteocephalus parallacticus and Eubothrium salvelini.

HISTORICAL REVIEW

The first report of an helminthic infection in the speckled trout in the Province of Quebec is that of Stafford who, in 1904, reported finding Distoma laureatum Zeder in trout brought to Montreal markets. The status of the species reported by Stafford was briefly reviewed by Miller in 1941 and is reviewed in more detail in this study. There was no further report of helminthic infection in this host until 1931, when Skinker (1931a) reported and described the nematode Metabronema canadense in trout from the Matamek River. In 1948 the writer (1948b) showed this species to be identical with Metabronema salvelini (Fujita, 1920). In 1933, Wardle reported finding Diphyllobothriid larvae in trout from the Ungava region. In 1936, Richardson reported on the parasitic infections of speckled trout in Lake Edward and also recorded as a new species, the nematode Philonema salvelini. Lyster in 1940 (1940a) reported on helminthic infections of trout in Lake Commandant and described three new species, namely: Ptychogonimus fontanus, Rhaphidascaris alius and Rhabdochona laurentiana. The last of these species was shown by the writer in 1951 to be synonymous with R. cascadilla. In 1940, Lyster

(1940b) also showed that the so-called "black spot" disease of trout in Quebee was caused by the metacercarial stage of a trematode which he described as the new species <u>Apophallus imperator</u>. Miller later (1941b) showed it to be synonymous with <u>Apophallus brevis</u>, the adult stage of which is known to occur in the loon (<u>Gavia</u> immer).

In 1947 the writer described a new species under the name of <u>Phyllodistomum lachancei</u>, a trematode found in the ureters of trout in the Laurentide Park, and in 1948 (1948a) recorded a general survey which he made on the internal helminths parasitic in trout in this area. In the same year Fantham and Porter (1948) reported on the parasitic infections of trout in various lakes and streams of the Gaspe Peninsula, Eastern Townships and the Laurentians, north of Montreal.

The species of helminths reported by the authors cited above as infecting speckled trout in the Province of Quebec are listed in Table 1. The literature for each species discussed in this thesis will be included in the section pertaining to that particular species.

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TABLE 1

PARASITIC HELMINTHS REPORTED FROM THE SPECKLED

TROUT IN THE PROVINCE OF QUEBEC

Species

Authors

Trematoda

Crepidostomum cooperi

Crepidostomum farionis

Crepidostomum cornutum

Clinostomum complanatum

Phyllodistomum lachancei

Apophallus imperator

(= A. brevis)

Pleurogenes sp.

Stafford, 1904; Richardson, 1936; Lyster, 1940a; Fantham and Porter, 1948; Choquette, 1948a.

Choquette, 1948a.

Fantham and Porter, 1948.

Lyster, 1940b.

Lyster, 1940a.

Fantham and Porter, 1948.

Fantham and Porter, 1948.

Choquette, 1947 and 1948a.

Fantham and Porter, 1948.

Ptychogonimus fontanus

Phyllodistomum superbum

Cestoda

Eubothrium salvelini

Diphyllobothriid larvae

Proteocephalid larvae

Richardson, 1936; Lyster, 1940a; Fantham and Porter, 1948; Choquette, 1948a.

Wardle, 1933; Richardson, 1936; Fantham and Porter, 1948; Choquette, 1948a.

Ligula sp. and L. intes- Fantham and Porter, 1948; tinalis. Choquette, 1948a.

> Lyster, 1940a; Choquette, 1948a.

Table 1 (Continued)

Nematoda

Metabronema salvelini (= <u>M. canadense</u>, <u>Cysti</u>-<u>dicola</u> harwoodi)

Rhaphidascaris alius

Philonema salvelini

Philometra sp.

Rhabdochona cascadilla (= B. laurentiana)

Agamospirura sp. innom.

Skinker, 1931a; Lyster, 1940a; Choquette, 1948a and 1948b.

Lyster, 1940a.

Richardson, 1936.

Fantham and Porter, 1948.

Lyster, 1940a; Choquette, 1948a and 1951.

Choquette, 1948a.

Acanthocephals

Neoechinorhynchus cylindratus Lyster, 1940a.

Echinorhynchus lateralis

Richardson, 1936; Fantham and Porter, 1948; Choquette, 1948a.

MATERIAL

Adult and larval worms used in these studies came from trout collected by the writer during the summer months from 1947 to 1953, and during the past fifteen years, by field parties from the Institute of Parasitology, by the Office of Biology, Quebec Department of Fish and Game, and by anglers in various parts of the province including: Laurentide Park, Mont Tremblant Park, Papineau, Argenteuil, Labelle, St. Maurice, Joliette, Montcalm, Laviolette, Champlain and Kamouraska counties, as well as from Knob Lake and Star Lake in Labrador. However, most of the material came from trout caught in lakes and streams of the Laurentide Park.

The Laurentide Park is situated in the Laurentians, between the forty-seventh and forty-eighth degrees of latitude at an altitude varying between one thousand to three thousand feet and its area covers more than four thousand square miles divided among four counties: Chicoutimi, Lac St. Jean, Charlevoix and Montmorency. This area is reserved largely for trout fishing, although some parts of it are fairly highly industrialized. Although it is believed that there are more than a thousand lakes in the Park (Vladykov (1942)) the exact number is not yet known and less than two

hundred of these lakes are open to fishing. There are two watersheds in the area: the Lake St. John - Saguenay River and the St. Lewrence River. There are seven drainage systems, namely: the Chicoutimi River, the Jacques Cartier River, the Montmorency River, the Metabetchouan River, the Belle-Riviere, the Malbaie River and the Ste. Anne du Nord River. The accompanying map (Fig. 1) shows the configuration of these systems. Fish were taken from forty-four lakes and streams including all seven drainage systems.

The trout used for experimental purposes were two to three year old parasite-free fish raised in hatcheries. They were kept at temperatures varying between 52-59°C in running water supplied by an artesian well. They were fed minced beef heart and liver but were starved for several weeks prior to their utilization for experimental purposes.

In studies of the life history of the kidney fluke, <u>Phyllodistomum lachancei</u>, clams of the family Sphaeriidae were collected from lakes and streams where fish were known to harbour the infection. Examination of these clams showed some of them to harbour the larval stages of another trematode, <u>Crepidostomum cooperi</u>. The clams were identified through the kindness of the Rev. H. B. Herrington, Keene, Ont., as species of the genus <u>Pisidium</u>.

In studying the life histories of trout helminths the aquatic stages of several species of insects were collected from the lakes and streams of the Laurentide Park and examined for the presence of larval Thus, damselfly naiads were found to harbour worms. the metacercarial stage of the kidney fluke while nymphs of a mayfly (Hexagenia recurvata) and of a species of the genus Polymitarcys harboured the larval stage of the trematode Crepidostomum cooperi and of the nematode Metabronema salvelini. Damselfly naiads used for experimental purposes during the course of study on the life history of the kidney fluke were collected along the shore of Lake St. Louis at Ile Perrot and of the Ottawa River at Ste. Anne de Bellevue and Dorion. Multiple examinations of these naiads failed to show the existence of any infection.

For the purpose of establishing the definitive hosts of the larval Diphyllobothriid found in the trout, several hosts, both avian and mammalian, were used in attempts to establish experimental infection. The birds used were down-covered ducklings (Mallard and domestic) a few days old and very young ring-bill gulls (<u>Larus</u> <u>delawarensis</u>) as well as day-old chicks. The ducklings, chicks and some of the gulls were hatched and raised in the laboratory. The gull eggs were hatched at a temperature of 103°F. During the period of incubation the gull

eggs were dipped in lukewarm water to prevent the young birds sticking to the shell. After hatching the gulls were left in the incubator for about twentyfour hours and then transferred to a brooder set at 100°F. for another twelve to fifteen hours. They were then transferred to a box kept at 75-80°F. The ducklings and chicks were fed parasite-free trout or thawed out smelt, cod, sole. Two adult herring gulls (<u>Larus argentatus</u>) kept in the Quebec Zoological Garden were also used.

The mammalian group included nursing kittens, hamsters and black bear cubs. The bear cubs were fed only bread soaked in milk and minced horse meat. A human volunteer (the writer) also served for experimental purposes.

IMPIIODS

Helminthological Material

Live and fixed specimens of helminths were used both for experimental nurposes and for morphological studies. Some of the trematodes and cestodes were killed by leaving them overnight in cold water; others by plunging them into a fixative. The flatworms were fixed in five per cent formalin or in Bouin's fixative. Some of the cercariae of the kidney fluke were fixed in hot lactophenol while others were fixed in five per cent formalin or in Bouin's fixative. Some encysted and mechanically excysted metacercariae of Phyllodistomum lachancei and of Crepidostomum cooperi from experimentally or naturally infected damselfly naiads or nymphs of species of mayfly were studied without staining, and others after staining with neutral red. Others were examined after fixation and staining with Alum carmine. Adult nematodes from trout and nematode larvae from numbhs of species of mayfly were fixed in five per cent formalin or hot seventy per cent alcohol. Alum carmine and Celestin blue were used in the staining of adult and larval trematodes and cestodes while nematodes were studied after being cleared either in lactophenol or glycerine. Vital stains used in a few instances did not prove satisfactory. Permanent mounts were made of whole worms

and parts thereof. The trematodes and cestodes were mounted in Canada Balsam while nematodes or parts of them were mounted in glycerine jelly.

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The Diphyllobothriid plerocercoids which were used for experimental purposes were dissected from their cysts found on the viscera of trout. Upon dissection the plerocercoids were kept in physiological saline solution in tubes placed in running water at a temperature of about 52-59°F. They were thus kept alive for about a week. Eggs of the kidney fluke, <u>Phyllodistomum lachancei</u>, were secured by dissecting the uterus of the adult worm. The eggs hatch shortly after being put in water. The miracidia were studied alive after staining with neutral red.

Molluscan Material and Larval Stages of Trematodes

Clams were collected in the Laurentide Fark; those used in the life history study of the kidney fluke were from a sandy beach in Grand Lac Carre, and those used in the biological studies of <u>Crepidostomum cooperi</u> from similar beaches at Lake Turgeon and the Ste. Anne du Nord River.

Clams were collected by sifting sand and muck taken nearby growing aquatic plants. The material left on the sieve was transferred to a tray and washed to remove plant debris and large particles. The remainder, if any

clams could be seen, was then transferred to a thermos bottle packed with crushed ice or to jars kept in a portable refrigerator and taken to the laboratory where the clams were individually sorted.

In the laboratory, the clams were separated in lots of about one hundred and placed in well water in Petri dishes. Every morning and evening each container was examined for cercariae under a 8x dissecting microscope. The cercariae were removed with the aid of a pipette. Some of the cercariae thus collected were used for experimental purposes and others were kept for morphological studies. In those dishes where cercariae were found the clams were further separated into smaller lots of twenty to thirty clams.

Difficulties were at first experienced in keeping the clams alive under laboratory conditions. In addition to starvation, one of the main causes of death appeared to be the development of fungal infections which seemed to develop faster in dishes containing several clams. In order to overcome this difficulty the clams were transferred daily into clean dishes. Bathing in fungicides was of no discernible help. Hand removal of fungi growing on the shell of the clams by gently rubbing between the fingers resulted in longer survival, but this proved to be a tedious and time-consuming task as several hundred clams were involved. Finally, it was found that by placing the

clams in an Erlenmeyer flask containing fine sand and a little water and shaking it vigorously for a minute or two, the shells of the clams were freed of fungi. This was repeated with the result that the mortality rate decreased rapidly and clams could be kept for several weeks. This technique of clam bathing avoids the task of setting up aquaria, while still permitting easy examination for cercariae.

Infected clams were crushed lightly between glass slides in order to obtain sporocysts. The sporocysts were fixed in either five per cent formalin or in Bouin and stained with Alum carmine.

Arthropod Material and Larval Stages of Helminths

The collection of clams by the method described above yielded mayfly and dragonfly nymphs. The collection of the naiads of damselflies, which cling to the stems of aquatic plants, was made with the help of a net devised by the writer. This net consists of a rim made of a solid steel rod one centimeter in diameter. The semicircular rim is eighteen inches at its widest and nine inches across. To this rim, wire window screening is fastened to make a basket about six inches deep. A handle about five feet long is attached to the net, which is used in sweeping the weeds. The contents of the basket are transferred to a

wide dish from which the naiads and other aquatic arthropods can be picked up.

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The damselfly naiads, mayfly and dragonfly nymphs, and other arthropods which were collected in areas where fish are known to harbour helminthic infections were taken to the laboratory in cooled containers. At the laboratory these arthropods were dissected and examined for the presence of larval helminths.

The naiads collected for experimental purposes were kept individually in small bottles placed in a wooden frame left floating in running water at about 52-59°C. Prior to their use the naiads were starved for several days, since non-starved naiads proved to be unsatisfactory feeders. The naiad to be fed cercariae of the kidney fluke was placed with the cercariae in a Syracuse dish thus permitting examination of the process of feeding under the dissecting microscope. When necessary, in order to encourage feeding, the cercariae were directed by means of a dissecting needle towards the naiad.

Experiments with Fish, Avian and Mammalian Hosts

Parasite-free trout were used for biological studies of the kidney fluke <u>Phyllodistomum lachancei</u>, the fluke <u>Crepidostomum cooperi</u> and the nematode <u>Metabronema</u> <u>salvelini</u>.

Fish used for experimental purposes were starved for several weeks. After exposure to experimental infection the fish were either kept in separate aquaria provided with an electrically driven aerator, or tagged and transferred to troughs. The fish were killed at various intervals and examined.

In studies on the development of Diphyllobothriid plerocercoids, the ducklings, chicks and gulls were infected shortly after their hatching, usually within forty-eight hours. Live plerocercoids were administered by means of gelatin capsules introduced into the gullet of the birds or into the pharynx of the mammals. Faeces of one of the bears were examined at weekly intervals during a period of two months following initial feeding of plerocercoids. Birds, the bears and the kittens were killed at various intervals and examined. In attempts to infect himself the writer on three occasions in 1948, 1949 and 1952 swallowed eight to ten plerocercoids; weekly faecal examinations were made during a period of three months.

Specific methods will be discussed under the experiments to which they applied.

TREMATODA

Three species of trematodes were investigated during the course of this study, namely: <u>Phyllodistomum</u> <u>lachancei</u>, family Gorgoderidae Looss, 1901, and two species of the genus <u>Crepidostomum</u>, family Allocreadiidae Stossich, 1904.

GORGODERIDAE Looss, 1901.

Diagnosis

Members of the family Gorgoderidae are small and are usually found with a smooth cuticle, but sometimes bear papillae. The body is generally divided into two portions: the anterior or pre-acetabular being slender and mobile, while the posterior or post-acetabular is broad and flattened. The oesophagus is short or long and the pharynx may be present or absent, while the intestine is single and not ramified. The genital pore is median and pre-acetabular. Male genitalia: the testes are usually oblique to each other, inter-caecal or extra-caecal; cirrus pouch and cirrus are absent; pars prostatica is feebly developed and vesicula seminalis is usually small. Female genitalia: the ovary is pre-testicular; vitellaria are paired and post-acetabular; the uterus is greatly folded with descending and ascending inter- or extracaecal limbs and with the coils tending to fill the posterior region of the body. The eggs are numerous. Adult worms are normally found in the urinary tract of fish, amphibians and

reptiles.

The family consists of two subfamilies: Gorgoderinae Looss, 1899, and Anoporrhutinae Looss, 1901. The Gorgoderinae are characterized by the absence of a pharynx, the presence of two to nine compact or lobed testes placed posterior to the usually compact vitellaria glands. The ovary is lateral and posterior to the vitellaria and slightly anterior to the anterior testis; the seminal receptacle is absent and Laurer's canal present. The subfamily at present contains the following genera: <u>Gorgodera</u> Looss 1899, <u>Gorgoderina</u> Looss 1902, <u>Phyllodistomum</u> Braun, 1899, <u>Xystretrum</u> Linton 1910, <u>Macia</u> Travassos 1922.

The Biology of the Gorgoderids

Although several species of Gorgoderidae from a variety of hosts have been described, data on their development are scanty. Sinitzin, in Europe (Goodchild (1943)), in 1905 was first to report on the life history of members of this group of trematodes. He studied the life cycle and described the larval stages of three species of the genus <u>Gorgodera: G. pagentcheri, G. cygnoides and G. varsoviensis</u> and of a species of the genus <u>Gorgoderina, G. vitelloba</u>. In all cases odonatan naiads and beetles served as second intermediary hosts. Sinitzin was able, upon experimental feeding of species of European Cyprinidae with metacercariae of a

form he considered to be <u>Phyllodistomum</u> folium, to recover excysted specimens in the intestine of these fish two hours after feeding, and young worms in the urinary ducts after twenty-four hours.

In 1926, Lutz, working in South America, reported that the cercaria of an unidentified Gorgoderid develops in clams of the genera <u>Cyclas</u>, <u>Pisidium</u> or <u>Sphaerium</u> and that it encysts in the cesophagus of the dragonfly nymph.

Krull (1933) (1935) (1936) studied the life cycle of <u>Gorgodera amplicava</u> Looss, parasitic in the urinary bladder of the frog. He showed that the cercaria of this fluke encysts in damselfly naiads or in the snail <u>Helisoma</u> <u>antrosa</u>. His studies were the first in North America on the life history of a gorgoderid trematode. Since that time Goodchild (1945) (1948) has contributed additional data on the life history of this fluke and on the morphology of its larval stages.

In 1938, Wu reported finding the progenetic metacercaria of <u>Phyllodistomum lesteri</u> in fresh-water shrimps in the Shanghai area but was unable to determine the host of the adult worm.

In 1939, Rankin in the United States determined the life history of <u>Gorgoderina attenuata</u>, another frog bladder trematode, and showed that tadpoles of the genus <u>Rana</u> are its usual secondary host. Crawford in 1939 and in 1940 reported that odonatan nymphs, caddice fly larvae and diving beetle

larvae act as second intermediary hosts of <u>Phyllodistomum</u> <u>americanum</u>. Goodchild (1940) (1943), also working in the United States, demonstrated the life history of <u>Phyllodistomum solidum</u> parasitic in salamanders. Both Goodchild and Groves (1945) reported that odonatan naiads are the second intermediary host of <u>P. solidum</u>. Goodchild's study is the first in which the life history of a species of the genus <u>Phyllodistomum</u> has been demonstrated. In 1945, Groves redescribed <u>P. solidum</u> and provided experimental proof of its life history. However, this author refers only to a 1940 abstract by Goodchild and not to Goodchild's 1943 paper. In 1950, Hunt reported on the development of an undescribed species of Gorgoderid parasitic in the urinary bladder of the frog Rana clamitans.

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As stated by Goodchild (1943), several European workers have postulated on morphological similarities and slight experimental evidence certain relationships between cercariae or metacercariae and sexually mature Phyllodistomes. Nybelin in 1926 (Goodchild (1943)) amplified these speculations concerning European Phyllodistomes. However, in no case was the life history of a Phyllodistome elucidated.

Studies on the biology of the Gorgoderids have brought forth several interesting observations pointing to the existence of two and three-host cycles among these forms. For example, <u>Cercaria</u> <u>duplicata</u> of Reuss (Goodchild

(1943)) upon leaving its bivalve host, becomes a metacercaria which sinks to the bottom of the water and lies dormant until it is taken by the next host. Another example of a two-host cycle is that observed by Sinitzin who reported in 1901 (Goodchild (1943)) that the cercaria of Phyllodistomum folium encysts in the parent sporocyst which then emerges from the bivalve and is eaten by the next host. Three-host cycles have been shown in a few species of Gorgoderids. Thus the cercaria of Gorgodera amplicava, according to Krull (1935), and the cercaria of Gorgoderina attenuata, according to Rankin (1939), are both ingested with food by snails and amphibian larvae in which they encyst as metacercariae. In 1948, Joyeux and Baer experimentally secured the encystment of an unidentified cercaria in tadpoles of Bufo vulgaris and Rana esculenta. Other examples of three-host cycles are those reported by Sinitzin in 1905 (Goodchild (1943)), Lutz (1926), Krull (1935), Goodchild (1940) (1943) and Crawford (1939) (1940).

In addition to Wu's 1938 report of finding progenetic metacercariae in fresh-water shrimps there is the one of Joyeux and Baer who in 1934 found bladder flukes in the musculature of <u>Rana esculenta</u>; these trematodes may also be precocious metacercariae. Thus it is evident that the intermediate hosts of these three-host cycles, although essential in the biology of the trematodes are, however,

only vectors which by acting as a source of food for the definitive host facilitate the completion of the life cycle.

Gorgoderid flukes are usually found in the urinary bladder and in the Wolfian ducts of the fish or amphibian hosts. According to Goodchild (1943), these worms probably feed on the urinary epithelium as evidenced by his finding of epithelial debris in the intestine of a speciman of <u>P. solidum</u>. However, their habitat is not limited to the bladder or ureters as shown by Joyeux and Baer (1934), Van Cleave and Mueller (1934), Odlaug (1937), Rankin (1939), Goodchild (1945) (1948), who found them in other parts of the host's body. Goodchild (1950), corroborating Odlaug's finding of immature forms of <u>Gorgoderina amplicava</u> in the mesonephric tissue of frogs, has shown that those worms which are unable to extract themselves from the kidney tissue become encapsulated and remain trapped there during their adult life.

Rankin in 1939, in his study on the life cycle of <u>Gorgoderina attenuata</u>, and Goodchild (1940) (1943) in his on the biology of <u>P</u>. <u>solidum</u>, have shown that the young worms after excystment in the small intestine migrate posteriorly along the mucosa of the large intestine to the cloaca from where they eventually reach the bladder and the ureters and, in the case of female hosts, the oviducts. However, the path of migration into the oviduct is unknown. How the young

worms orient themselves to the proper cloacal opening is unknown. Goodchild (1943) is of the opinion that in the salamander <u>Desmognathus fuscus fuscus</u> orientation of the growing <u>Phyllodistomum solidum</u> may be provided by the action of host cloacal ciliae. In an experiment cited above, Sinitzin reported recovering excysted specimens of Phyllodistomes in the intestine and urinary tract of fish fed metacercariae. However, the number of worms recovered by Sinitzin was much smaller than the number of metacercariae fed. This author suggested that the young worms migrated from the anal opening to the urinary tract opening and that the hazards of such a migration would explain the lesser number of worms found in the urinary ducts.

Genus Phyllodistomum Braun, 1899

(Syn.: <u>Spathidium</u> Looss, 1899, <u>Catoptroides</u> Odhner, 1902, <u>Microlecithus</u> Ozaki, 1926, and <u>Dendrorchis</u> Travassos, 1926)

Systematic Status

The systematic status of the genus <u>Phyllodistomum</u> Braun, 1899, of the family Gorgoderidae Looss, 1901, subfamily Gorgoderinae Looss, 1901, has been discussed by several students of the genus and particularly by Lewis in 1935. This genus was created by Braun to include all the then known species of trematodes from the urinary bladder of different

cold-blooded vertebrates into a single genus with <u>Distomum</u> <u>folium (= Phyllodistomum folium</u> (V. Olfers, 1817) (Braun, 1899)) as type species. Following Braun's creation of the genus <u>Phyllodistomum</u> four other genera were created to include such trematodes. These genera: <u>Spathidium</u> Looss, 1899, <u>Catoptroides</u> Odhner, 1902, <u>Microlecithus</u> Ozaki, 1926, and <u>Dendrorchis</u> Travassos, 1926, were eventually, as pointed out by Goodchild (1943), shown to be synonyms of Braun's genus. Since then several species of trematodes parasitic in the urinary tract of cold-blooded vertebrates have been added to this genus.

Generic Diagnosis

Gorgoderinae: the body is usually spatulate and the cuticle smooth or with spines. The body is often notched in its posterior region. The oral sucker is usually smaller than the acetabulum. The pharynx is absent and the oesophagus of varied length. The genital pore is usually between the intestinal bifurcations and anterior margin of the acetabulum. Testes: there are two slightly or deeply lobed, slightly oblique. The vas deferens is long and the seminal vesicle is conspicuous while the prostate and the ejaculatory duct are inconspicuous. The ovary is compact or slightly lobed and usually posterior to the vitellarium of the same side but is pre-testicular and may be amphitypic. The oviduct is relatively long and the fertilization space

generally evident. Laurer's canal is present, paralleling the vitelline duct on the side opposite to the ovary. The Mehlis gland is present but inconspicuous. The metraterm is large and vitellaria are compact or lobed, postacetabular but pre-ovarial. The eggs are numerous filling many uterine coils. The excretory pore is subterminal and the excretory vesicle is Y-shaped. Type species: <u>Phyllodistomum folium</u> (V. Olfers, 1817) Braun, 1899. Synonyms: <u>Distoma folium</u> V. Olfers, 1817 and of Rudolphi 1819 and Looss, 1894; <u>Spathidium folium</u> (V. Olfers) Looss, 1899; <u>Phyllodistomum pseudofolium</u> Nybelin, 1926.

Phyllodistomum lachancei Choquette, 1947

Habitat, Distribution and Incidence.

This trematode, described by the writer in 1947, occurs in the ureters and the urinary bladder of the speckled trout. The flat edges of the worm being unrolled, it practically occludes the lumen of the ureter and, to the naked eye, presents the appearance of a small abcess. The worms were found indiscriminately in either the left or the right ureter in unilateral infection while a few fish were found to be bilaterally infected. The worms are removed easily from their habitat by gently pressing along the length of the ureter in the direction distal from the kidney.

The parasite was found in trout from lakes and streams of the Jacques Cartier River, Malbaie River and the Ste. Anne du Nord River drainage systems of the Laurentide

These are the only localities where the infection Park. has been recorded. Fantham and Porter in 1948 reported finding a single specimen of Phyllodistomum which they identified as P. superbum from the gall bladder of a trout from Lake Memphremagog. Unfortunately these authors did not elaborate their finding and the writer was unable to secure this specimen for study. This is very likely a case of accidental parasitism by a form parasitic in the urinary tract of Esocidae known to inhabit these waters. Until more is known of the helminthic infections of trout and Esocidae in this locality, this report must be regarded as doubtful. In view of available knowledge of the helminthic infections of the speckled trout in North America, one may infer that P. lachancei is actually the only species of the genus which so far is known to occur in the urinary tract of this host. And the Province of Quebec is so far the only locality where it has been recorded.

While statistics on the incidence of intestinal worms are relatively easily established through the collection of viscera by fishermen, guides, cooks, etc., it is not easy to secure kidneys as these are usually removed at the time of evisceration. The removal of the kidney is usually done with the help of a knife and the organ is mutilated and reduced to a bloody pulp so as to make it almost impossible to find worms. However, on some occa-

sions during the summers of 1950 and 1952 the writer examined the kidneys of a number of trout from lakes and streams of the Ste. Anne du Nord River drainage system. One hundred and forty fish were thus examined and sixtysix, or forty-seven per cent, were found to harbour the parasite. The highest incidence was encountered in fish from Lake Turgeon, where the fluke was found in forty-nine of eighty-two trout. In trout from lakes of the Malbaie River drainage system the incidence is almost fifty per cent.

Life History Studies

Experiments with second intermediary hosts.

Naiads of various species of damselfly were offered cercariae. Naiads were fed one to five cercariae. In some instances the offered cercariae were very active; in others they were sluggish and seemed to be less challenging to the naiads. The very active cercariae were usually attacked immediately, while with the sluggish ones a needling was often needed.

In the process of feeding, the naiad projects its elongated labium and grasps the cercaria, pulling it into contact with the open jaws which direct it into the mouth. Naiads usually ingested the cercariae in a few seconds, especially if the cercariae were lively and were grasped by the anterior extremity. In those cases where

the cercariae were grasped by the posterior extremity or the middle of the body, they were often not swallowed and were rejected after being held for a few minutes. That some of the cercariae failed to encyst themselves in the insect host is evidenced by the fact that in some of the naiads known to have ingested cercariae, no cysts, or a smaller number than were expected, were found upon examination. Goodchild (1943) thinks that such failure could be due in some cases to imperfect cercariae. A1though this is possible, the writer also believes that this failure is due to physical damage to the cercariae during their passage through the mouth structures. The cercariae penetrate the crop wall shortly after being ingested, and encyst in the walls of the thorax. This process of encystment of the cercaria is readily watched under the dissecting microscope, especially if the naiad is light in colour.

Sinitzin (Goodchild (1943)), Krull (1935) and Goodchild (1943) report that the encystment is a cause of discomfort to the insect, as shown by the rubbing of the legs together or the body with the legs, nervous wriggling movements and short random dashes through the water, loss of equilibrium and tetanosis. The writer has had the opportunity of observing many feeding naiads, and has seen naiads remain completely motionless after having ingested cercariae while others, which had not been fed, acted in

the manner described by Goodchild. However, while some naiads would ingest only one cercaria in from one half to several hours, others would ingest two, three or four within a minute or two of exposure. The metacercaria wall which appears as a delicate membrane was formed shortly after penetration and within the hour, the cyst was completely formed.

Experiments with the definitive host.

Three three year old trout were used for experimental infection of <u>Phyllodistomum lachancei</u>. Two methods of infection were tried.

One of the trout was fed metacercariae which had been dissected within ten to fifteen minutes of feeding from experimentally infected damselfly naiads. The fish was held enveloped in a wet towel and the metacercariae were forced into its stomach with the help of a rubberbulb glass pipette with a smooth tip. Ten metacercariae three to six days old were thus introduced. The fish was then returned to an individual aquarium. However, it could not be determined whether the metacercariae were regurgitated. The trout was killed three days after initial feeding and examination of the urinary ducts showed no sign of infection.

Two of the trout were fed naiads which were

known to harbour metacercariae. In order to determine whether the naiads harboured metacercariae, they were placed between two glass slides and gentle pressure applied; examination was thus possible under the dissecting microscope. The metacercaria can be seen with reflected light because of the presence of opaque concretions in the excretory bladder. The naiads were introduced into the stomach with the help of forceps in one case and of a large rubber-bulb glass pipette in the other. After feeding, the trout were returned to individual aquaria and observed for signs of regurgitation. One of the trout was thus fed sixty-one naiads harbouring ninety-eight metacercariae seven to ten days old; the other was fed fifty naiads harbouring seventy metacercariae two to six days of age. One of these trout was killed twenty-four hours after initial feeding and eight excysted forms were found in the terminal portion of the intestine and in faeces covering the anal opening and urogenital sinus. There was an indication that the excysted worm had undergone a physiological change. Metacercariae mechanically excysted live only about fifteen to twenty minutes at most, whereas the naturally excysted form remained alive in tap water and faeces for three to four hours after recovery from the trout. However, no gross morphological development was The other trout was killed fourteen days after observed. initial feeding, but no worms were found on examination.
Discussion of life history

The studies of Krull, Rankin, Crawford, Goodchild, Groves and Hunt are the only ones to date on the life histories of Gorgoderid trematodes parasitic in North American hosts, and their studies were on trematodes from amphibians. The present study is the first on the life history of a Phyllodistome parasitic in a fish host on this continent.

Examination of clams has shown that the first larval stages develop in bivalves of the genus Pisidium: P. ferrugineum Prime, P. obtrusale C. Pfeiffer, form ventricosum Prime and P. subtruncatum Malm. Experimentally, the writer has shown that the cercaria encysts in damselfly naiads and that excystment takes place in the digestive tract of the trout, from whence the young worms reach the urinary system. Further evidence that the worm must pass through the alimentary tract is indicated by the life span of the mechanically excysted larvae (fifteen to twenty minutes) as opposed to the naturally excysted worms (three to four hours). The change of physiological adaptation to a new environment appears to take place before the worm reaches its final destination, the urinary ducts. The writer considers this to be further evidence of the migration from the alimentary tract to the urinary system as opposed to a somatic or visceral

migration. As stated above, Gorgoderid trematodes are usually found in the bladder or ureters but as shown by Van Cleave and Mueller (1934), Joyeux and Baer (1934), Odlaug (1937), Rankin (1939) and Goodchild (1945) 1948), they also can be found in other parts of the body. However, in the present study <u>P. lachancei</u> was found only in the urinary bladder or the ureters of naturally infected fish, and then only in small numbers, usually not more than eight to ten in any one fish.

The data thus secured in the present study show that P. lachancei has a three-host life cycle. In this it is similar to some species of Gorgoderids. The data secured from experimental infection also point to the fact that many of the forms, after their excystment in the intestine, do not reach their final destination. This most probably is due, as suggested by Sinitzin (Goodchild (1943)), to their disappearance along their migratory path from the digestive tract to the urinary ducts of the host. In addition to the hazards of migration, certain physiological conditions which are present in fish in nature may be lacking in trout reared artificially. Were the fish kept on a diet similar to that which is available to them in nature, it is possible that the kidney fluke might become established more readily.

Description of Stages

1. <u>Miracidium</u> (Figures 2, 3)

Ripe eggs isolated from the metraterm contain fully developed miracidia which can be seen surging back and forth. These eggs hatch within a minute of being placed in tap water. This finding is not in agreement with Crawford's observation (1940) to the effect that for <u>Phyllodistomum</u> sp. there is a two-day interval between egg deposition and hatching. Upon hatching the miracidium swims about vigorously for several hours, but this activity decreases with time and only a few specimens were still alive after eighteen hours.

Due to contraction and extension, the shape of the miracidium is quite variable. At times the miracidium when quiescent is oval in shape or assumes a pyriform shape; it can also become elongated and show a constriction in its middle. It measures (oval form) 60-64µ in length by 50-54µ in width but at times it can be longer and narrower. The surface is covered with long ciliae about 6µ long, each of them possessing a distinct basal granule. They are lacking on both tips of the anterior and posterior extremity. The ciliae are borne on epidermal cells deeply stained with neutral red and filled with refractile granules. According to Rankin (1939) and several works quoted by him, these cells, after penetration of the miracidium into the clam's gills, are sloughed off and the sub-epithelium appears to become the wall of the mother sporocyst.

Internally the body is filled with many fat-like droplets. It is provided with a sac-like structure, the so-called "gut" which measures about 20µ by 14µ and appears as a clear empty space with a small opening facing the tip of the anterior extremity. Posterior to it are two glands usually laterally placed, in apposition to each other. These glands, easily seen in neutral red stained specimens, are quite variable in their size but one is always much larger than the other, the larger one being 35µ long by 17µ. In several specimens it was possible to see a narrow isthmus connecting both glands. Two flame-cells are present at about the level of the demarcation line between the middle and posterior third of the body but their excretory duct could not be made out satisfactorily.

2. Sporocysts (Figures 4, 5, 6)

Both mother and daughter sporocysts can be seen in infected clams. Immature mother sporocysts are tubular in shape and measure 0.59 to 1.0 mm. in length by 0.8 mm. in width; they are filled with germ balls. Ten to fifteen of these germ balls, themselves filled with secondary masses, can be seen. From these will arise the future daughter sporocysts. The sporocyst wall is 12µ to 15µ thick

and in it large cells can be seen.

The daughter sporocysts are pyriform and lie between the gill plates. The narrow anterior extremity is embedded in the gill tissue and its rounded posterior extremity is free and lies into the interlamellar gill space. The mature daughter sporocysts are 0.6 to 1.0 mm. long and 0.098 to 0.153 mm. wide at the anterior end and 0.267 to 0.520 mm. at the posterior extremity. The wall of the daughter sporocyst is 15µ to 20µ thick and contains many large cells. Internally the daughter sporocyst contains developing cercariae at different stages as well as a few germ balls. The number of cercariae is variable but four to six or seven were usually present in the sporocysts examined. The birth pore situated on the contracted anterior end was observed in one specimen in which the tail of a cercaria was seen protruding through it.

3. <u>Cercaria</u> (Figures 7, 8)

The cysticercous cercaria somewhat resembles that of <u>P</u>. <u>solidum</u> as described by Goodchild (1943) and Groves (1945) and that of an unknown Gorgoderid described by Joyeux and Baer (1948).

The cercaria upon being shed is very active but this activity decreases within a few hours, and twelve to twenty-four hours after being shed, the cercaria is usually inactive and extended. The mature cercaria

measures between 1.3 to 1.8 mm. in length and it consists of an anterior chamber containing the body of the cercaria and a posterior chamber or tail proper. The anterior chamber measures between 0.13 to 0.21 mm. in length. The tail is transparent with large cells scattered in its walls. In the immature cercaria the tail is short and thick and the body of the cercaria is not yet enclosed in the anterior chamber. In the mature cercaria the tail is elongated and the cuticle is raised into a circular fold to form the anterior chamber; this contains the cercarial body while the remainder forms the posterior Anteriorly, the cercarial chamber communicates chamber. with the exterior by a small pore; posteriorly, this chamber communicates with the tail proper by means of fibres extending from the lips of the excretory pore to a mass of cells situated at the proximal end of the tail proper. Both Goodchild (1943) and Groves (1945) report the presence of such cells in the cercaria of P. solidum. Groves is of the opinion that the structure extending from the excretory pore to these cells is a canal and that the cavity of the tail serves as an accessory bladder. This canal fits the description of Joyeux and Baer (1928) "lorsque la cercaire est sortie de sa chambre d'invagination, elle ne tient aux parois de celle-ci que par un mince pédicule qui se rompt facilement". Examination of immature cercariae of P. lachancei shows that there exists such

a "canal" leading from the excretory pore to the tail. Therefore, in view of this morphological arrangement, the writer shares Groves' opinion that in Gorgoderids the cavity of the tail acts as an accessory bladder.

The cercarial body is elongated and filled with a dense parenchyma which renders it difficult to secure a clear picture of the internal anatomy. Measurements vary in accordance with whether living or fixed material is used. Fixed specimens vary between 0.023 to 0.026 mm. in length, while living specimens may have greater length. Sensory papillae were seen in living specimens but cannot be discerned in stained material. These papillae were distributed as follows: fifteen to sixteen on each side of the body, four on the anterior extremity, extending between the anterior tip of the cercaria to a point level with the posterior margin of the oral sucker, four between the posterior margin of the oral sucker and the anterior margin of the acetabulum, one at the level of the acetabulum and five or six between the acetabulum and the posterior extremity.

The oral sucker, oval in shape, measures between 35-48µ in length and 41-48µ in width. The acetabulum, placed slightly behind mid-body, is 48-51µ long and 48-51µ wide while it is still larger in some specimens. The oral sucker opening is usually crescent shaped, while the acetabular one is either spherical or oval depending on the

amount of pressure applied to the preparation. The stylet embedded in the parenchyma dorsal to the oral sucker is 19 to 22µ long and between 4 to 5µ wide. Its anterior extremity is pointed; its posterior extremity is rounded; on each side there is a pair of lateral wings whose width decreases progressively toward the posterior end.

The ducts of the penetration glands situated about half way between the oral sucker and the acetabulum open on each side of the stylet. The penetration glands are better seen in neutral red stained preparations. The digestive tract is difficult to observe clearly. The narrow oesophagus bifurcates in front of the acetabulum, and the poorly developed intestinal caeca taper posteriorly towards the posterior extremity. The nerve ring appears in stained specimens as a transparent area across the cesophagus about midway between its bifurcation and the oral sucker. The ramifications of the excretory systems are difficult to follow. The flame-cell pattern appears to be complex and is difficult to follow owing to the refractile granules filling the body of the cer-Only a few flame-cells were seen, especially in caria. the posterior region of the body. The excretory bladder is well developed and is surrounded by a mass of large glands which extend anteriorly close behind the acetabulum. Anteriorly it receives the transverse branches

of the lateral excretory tubules extending anteriorly and posteriorly. The genital anlage could not be made out at all.

4. The Metacercaria (Figure 9)

In infected naiads secured between two glass slides the metacercaria can be seen with the aid of the dissecting microscope because of the opaqueness of concretions in the excretory bladder. The cyst is oval to spherical in shape and loosely attached to the thoracic walls; in it the active larva can be seen surging back and forth. The cyst wall of cercarial origin is about 3 to 4µ thick and in older cysts cellular elements of host origin adhere to it. The size of the cyst varies with its age. Thus cysts ten to fifteen days of age measure between 150µ to 175µ in their longest dimensions, while cysts about twenty-five days old measure between 190µ to 205µ.

In living cysts the suckers are easily seen, along with clusters of refractile granules, filling the excretory bladder. The cercarial stylet has become detached and can be seen free in the cyst cavity. The young worm is easily excysted, but it is very fragile and dies shortly after, its survival time not being more than fifteen to twenty minutes. A twenty-four hour metacercaria was 0.24 mm. long; the oral sucker was 41µ long by 41µ wide and the acetabulum 48µ by 48µ. A twelve day old metacercaria was 0.278 mm. long, the oral sucker was 57µ by 57µ and the acetabulum 60µ by 67µ while a twenty day old metacercaria was 0.352 mm. long by 0.131 mm. wide at the level of the acetabulum, with the oral sucker measuring 64µ by 55µ, and the acetabulum 60µ by 70µ. Examination of living specimens stained with neutral red showed that there is no improvement in the development of organ systems with age as seen in the cercaria, and that the penetration glands and their ducts have disappeared. Examination also failed to show the presence of the sensory papillae seen in the cercarial stage. This is not in agreement with Goodchild (1943) who reported that sensory papillae seen in the cercaria of P. solidum remain in the encysted stage.

5. The Adult (Figure 10)

The body consists of two portions: an anterior one, elongated and distinctly set off from a wider, spatulated posterior. The margins are smooth and the posterior end is notched. The average length of mounted specimens is 2.94 mm., while the average width taken at the level of space between the anterior margin of the posterior testis and posterior margin of the anterior testis is 1.31 mm. The terminal oral sucker is smaller than the acetabulum situated at the junction of the anterior and posterior portions of the body. The ratio of the diameter of the oral sucker to that of the acetabulum is as 1: 1.24.

The length of the oesophagus is less than the length of the oral sucker. The intestinal caeca extend to the posterior of the body. The vitellaria are oval and smooth, although occasionally, in immature specimens, indented vitellaria are seen; they are posterolateral and very close to the acetabulum, sometimes overlapping it. The vitelline ducts join medially. The shell gland lies between the vitelline glands in front of the common vitelline duct. The ovary is irregularly shaped, strongly lobate or faintly indented on either side, amphitypic; it is smaller than either testis and lies very close to the vitellarium of the same side. The uterus is voluminous and contains a great number of eggs; the uterine coils, intra- and extra-caecal, run backwards to the posterior margin of the body and forward to the anterior margin of the ovary. The testes are obliquely placed, fairly close to each other, elongated or roundish, irregularly lobate or indented. The distance between the testes is less than the lesser dimension of either testis. The testes lie in the middle of the posterior part of the body, the anterior one being behind and oblique to the ovary. The seminal vesicle is quite large and dilated posteriorly. The genital pore is situated ventrally in the space between the

intestinal bifurcation and the anterior margin of the acetabulum. The excretory pore is terminal and lies at the posterior notch.

Measurements are given in millimetres, with the average in parentheses, of 25 carmine-stained specimens, mounted in Canada balsam. Body: length, 2.01-3.68 (2.92); width, at level of the space between the posterior margin of the anterior testis and the anterior margin of the posterior testis, 0.96-1.49 (1.31). Oral sucker: diameter, 0.22-0.36 (0.278); acetabulum: diameter, 0.24-0.42 (0.346). Oesophagus: length, 0.105-0.181 (0.144). Right vitellarium: length and width, 0.07-0.22 by 0.10-0.27 (0.15 by 0.19); left vitellarium: length and width, 0.07-0.18 by 0.12-0.25 (0.13 by 0.19). Ovary: length and width, 0.16-0.39 by 0.15-0.36 (0.27 by 0.23). Testes -anterior: length and width, 0.31-0.74 by 0.19-0.54 (0.50 by 0.41); posterior: length and width, 0.36-0.75 by 0.30-0.57 (0.53 by 0.42); distance between anterior and posterior testes, 0.06-0.45 (0.18). The older intrauterine eggs obtained by dissection of the worm measure 0.03-0.05 by 0.02-0.03 (0.04 by 0.02).

Comparison of this species with descriptions of all the other known species of <u>Phyllodistomum</u> shows that it differs from all of them by the presence or absence of one or more morphological characters. By having a body

definitely divided into two portions it differs from P. <u>acceptum</u> (Lewis (1935)), <u>P</u>. <u>coatnei</u> (Meserve (1943)), <u>P</u>. conostomum (Lewis (1935)), P. elongatum (Lewis (1935)), <u>P. lesteri</u> (Wu (1938)), <u>P. linguale</u> (Lewis (1935)), <u>P</u>. <u>mariunum</u> (Lewis (1935)), <u>P</u>. <u>nocomis</u> (Fischthal (1942)), P. parasiluri (Yamaguti (1934)), P. sinense (Wu (1937)), An oral sucker smaller than the acetabulum distinguishes it from P. brevicecum (Steen (1938)), P. caudatum (Steelman (1938)), <u>P</u>. <u>etheostomae</u> (Fischthal (1943)), <u>P</u>. <u>mor</u>gurndae (Yamaguti (1934)), P. pearsei (Holl (1929)) and P. unicum (Lewis (1935)). The size, shape, and position of the gonads distinguish it from P. almorii (Pande (1937)), P. americanum (Lewis (1935)), P. angulatum (Lewis (1935)), P. carolini (Holl (1929)), P. entercolpium (Lewis (1935)), <u>P. fausti</u> (Lewis (1935)), <u>P. hunteri</u> (Arnold (1934)), <u>P</u>. <u>kajika</u> (Bhalareo (1937)), <u>P</u>. <u>lacustri</u> (Lewis (1935)), <u>P</u>. <u>lohrenzi</u> (Loewen (1935)), <u>P</u>. <u>lysteri</u> (Rankin (1932)), P. macrobrachiola (Yamaguti (1934)), P. macrocotyle (Lewis (1935)), P. megalorchis (Lewis (1935)), P. nocomis (Fischthal (1942)), P. notropidus (Fischthal (1942), P. patellare (Lewis (1935)), P. pseudofolium (Lewis (1935)), P. <u>semotili</u> (Fischthal (1942)), <u>P. shandrai</u> (Bhalareo (1937)), P. singulare (Lynch (1936)), P. solidum (Rankin (1937)), P. spatula (Lewis (1935)), P. spatulaeforme (Lewis (1935)), P. staffordi (Lewis (1935)), and P. undulans (Steen (1938)).

It resembles most closely P. folium (Lewis (1935)) and P. simile (Lewis (1935)). It differs from the former in length and width, size of oral sucker and acetabulum, shape and position of the testes, the extent of the uterine coils and size of eggs; it differs from the latter by its size, its larger vitellarium, the shape and position of the testes and size of eggs. Its smooth margins distinguish it from P. superbum (Lewis (1935)) and from P. lewisi (Srivastava (1938)). Therefore, it is considered that these differences are sufficient to separate the present specimens from the species described previously in this genus, and accordingly the name Phyllodistomum lachancei is proposed for it. Host: Salvelinus fontinalis (Mitchill). Habitat: Ureters and urinary bladder. Locality: Montmorency County, Province of Quebec, Canada. Type and co-types: British Museum (Natural History), Cromwell Road, London, S.W. 7, England.

Epidemiology

Invertebrate Hosts

Mollusca

As stated above, the following species of clams collected in Grand Lac Carré were found to harbour the cysticercous cercaria: <u>Pisidium ferrugineum</u> Prime, <u>P. ob-</u> <u>tusale</u> C. Pfeiffer, form <u>ventricosum</u> Prime and <u>P. subtrun</u>-

catum Malm.

The rate of infection of the clams collected during August and September was found to be between two and three per cent. Joyeux and Baer (1948), in their studies on the epidemiology of a Gorgoderid cercaria from <u>Sphaerium corneum</u> in southwestern France, found a relationship between the size, i.e. the age of the clam, and the index of infection. This is to be expected as with increased longevity the chances of infection are that much increased. This was evident in the present study as only those clams measuring between two to four millimetres were infected.

Joyeux and Baer (1948) also reported a seasonal incidence whereby under natural conditions the first sign of infection appeared in the spring, reached a maximum in the summer, decreased in the autumn and disappeared in the winter. According to these authors this observation conflicts with that made under laboratory conditions, where infection persists throughout the year. Hopkins (1934) in his studies on the life cycle of <u>Crepidostomum cooperi</u>, the larval stages of which are also found in bivalves identical or closely related to those harbouring the larvae of <u>Phyllodistomum lachancei</u>, has shown that the rate of infection is lower during the summer months than at any other time. This seasonal variation could be due to a

biological character inherent in <u>C</u>. <u>cooperi</u>. On the other hand, Goodchild (1943) in his studies on the life cycle of <u>P</u>. <u>solidum</u>, parasitic in salamanders, is of the opinion that the clams become infected in early spring and do not give off cercariae before the first of July. Unfortunately, due to climatic conditions prevailing in the area under investigation before June and after September, it was not possible to determine whether seasonal variations occur in the rate of infection of clams with the larval stages of <u>P</u>. <u>lachancei</u>.

Arthropoda

The aquatic larvae of many species of insects were examined. Among these were species of dragonfly, damselfly and ephemerids. Several hundred samples of each were examined for the presence of metacercariae. The dragonfly nymphs examined were found to harbour no metacercariae. However, a metacercaria identical to those secured from experimental naiads was found in two of the two hundred and forty-five damselfly naiads examined. The infected naiads were found in weeds growing on a beach near Grand Lac Carré, adjacent to where positive clams were found. Other larval aquatic arthropods and <u>Gammarus</u> sp. collected on the same spot were also examined and found negative.

Vertebrate Hosts

As stated above the worms are present in small

number, usually not more than eight to ten being found in any one fish. The finding of both sexually mature and immature worms in the same fish suggests their becoming infected any time during the summer months. This is plausible in view of the diet of the fish, which consists mostly of larvae of aquatic insects, particularly those of the damselfly. On the other hand, fish caught during the summer months from streams, (e.g. from the River Ste. Anne du Nord), do not usually harbour immature forms. This is accounted for by the fact that these fish do not have access to damselfly naiads, at least not to the same extent as fish in lakes; the writer believes that fish in streams acquire their infection when they migrate to the lakes at spawning time.

Pathology of P. lachancei

The only references available on the pathology of Gorgoderids are those of Choquette (1947) on <u>P. la-</u> <u>chancei</u> and of Goodchild (1950) on <u>Gorgoderina attenuata</u> and <u>Gorgodera amplicava</u> in the mesonephros and urinary ducts of frogs.

Goodchild studied the end of these juvenile forms which become imprisoned in capsules of host origin in the mesonephric tissue or mesonephric tubules which they reach from the ureters. According to this author, massive Gorgoderid infection in the renal tissue

and of the Wolffian ducts is detrimental to the host and in severe infection death may result. Goodchild reports that in such cases the kidneys are hyperemic and purplish and without doubt traumatized because of the heavy influx of flukes. In Goodchild's opinion death may be due to injurious histolytic wastes or the uremia caused by kidney failure.

The effect of very heavy infection by <u>P</u>. <u>lachancei</u> on the speckled trout has not been determined. The writer had the opportunity to examine a great number of fish but could not establish any relationship between the size of the fish and the presence or absence of the worm. Infection was noted in undersized trout in Lake Turgeon and in apparently normally developed trout in other lakes. On the other hand, no worms were found in other fish similar in every respect. As stated elsewhere, the number of worms present in any fish is usually quite small, and worms were usually found in the ureters with none being found imprisoned in the renal tissue.

However, this negative finding of severe infections, resulting in serious damage to the kidney, does not exclude such infections under natural conditions. Goodchild (1943) states that frogs heavily infected by bladder flukes are sluggish and sickly; there is, therefore, the possibility of heavily infected fish not being available for examination, as sickly fish would not readily take the

bait. There is also the possibility in fish of the existence of an immunity, limiting worms from becoming established, although Goodchild's observation in cases of severe infection of frogs by bladder flukes seems to exclude the possibility of an immunity developing in a host harbouring Gorgoderids.

Sections (Figure 11 (a, b, c, d)) of the kidney of fish harbouring the average number of worms usually found were made, showing that the presence of the parasite in the ureters produces at most only slight pathological changes. There is a dilatation of the ureter with some flattening of the lining columnar epithelial cells. A study of these sections shows no evidence of damage to the parenchymal tissues.

ALLOCREADIIDAE Stossich, 1908

In the present study this family is represented by two species of the genus <u>Crepidostomum</u> Braun, 1900, namely: <u>C. cooperi</u> Hopkins, 1931, and <u>C. farionis</u> (D. F. Müller, 1784). Both species are small and possess a smooth cuticle and well-developed suckers, the oral one with anterior processes. The presence of these anterior processes is specific to the genus <u>Crepidostomum</u>.

Crepidostomum cooperi Hopkins, 1931.

Distribution in Speckled Trout in Quebec

Of the two species of <u>Crepidostomum</u> found in the speckled trout in Quebec, <u>C</u>. <u>cooperi</u> is by far the more common form encountered. It was found in 107 out of 210 fish from the Laurentide Park in all the seven drainage systems of the area. It was also found in trout from lakes of the Mont Tremblant Park area and in Argenteuil, Labelle, St. Maurice and Champlain counties.

The earliest report of this species in the speckled trout in this country is that of Stafford who, in 1904, recorded it in fish from this province (vicinity of Montreal) and from New Brunswick under the name of <u>Distoma laureatum</u>. It was subsequently recorded by Richardson (1936) under the name of <u>Crepidostomum fausti</u> Hunninen and Hunter, 1933, from trout from Lake Edouard and Lake Ecarte (Champlain County), by Lyster (1940a), who found it in trout from lakes in Labelle and Papineau counties, and by Fantham and Porter (1948), who found it in trout from the Gaspé Peninsula.

These constitute the only records of this species in the speckled trout in Canada, although it has been reported in many instances from this host in the United States. While <u>C. cooperi</u> has been recorded by Bangham (1940) and Bangham and Venard (1946) in other species of fish in lakes of the Algonquin Park, Ontario, it has yet to be reported from the speckled trout in these localities.

The systematic status of Stafford's Distoma laureatum Zeder, 1800

As stated above, <u>C</u>. <u>cooperi</u> has already been reported from the speckled trout in this country by Stafford (1904) and by Richardson (1936) under other names, namely: <u>Distoma laureatum</u> and <u>Crepidostomum fausti</u>. While the species reported by Richardson as <u>C</u>. <u>fausti</u> has been shown by Hopkins (1934) to be identical with <u>C</u>. <u>cooperi</u>, the status of the form described by Stafford has not been satisfactorily established.

In Nicoll's opinion (1909) the American form reported by Linton (1893) from <u>Salmo</u> <u>mykiss</u>, and by Stafford from the speckled trout under the name of <u>D</u>. <u>laureatum</u>, although identical with each other, are not identical with the European form bearing the same name (<u>D</u>. <u>laureatum</u> Zeder,

1800 (= Crepidostomum laureatum (Zeder) Braun, 1900). Thus, Nicoll named the American form Stephanophiala transmarina, which he incorporated, along with the European form of <u>Crepidostomum</u> farionis (Müller, 1784), into his new genus Stephanophiala. However, at a later stage Nicoll (1924) admitted Stephanophiala to be identical with Crepidostomum Braun, 1900, and Faust (1918) and Hopkins (1934) showed S. transmarina to be identical with C. farionis (Müller. 1784). In 1933, Hunninen and Hunter (1933) described specimens they collected from trout in New York as Crepidostomum transmarinum (Nicoll), claiming them to be different from the American form of Crepidostomum farionis. However, the opinion of Hunninen and Hunter is not held by Hopkins (1934), who maintains that C. transmarinum is identical with C. farionis. As pointed out by Hopkins (1931). Stafford's report of C. laureatum from the speckled trout and other hosts has often been cited together with that of Cooper (1915) on this species. As shown by Hopkins (1931), Cooper's C. laureatum is not identical with C. laureatum, which has been shown (vide supra) to be identical with C. farionis. Hopkins (1931), after studying Cooper's C. laureatum, found it to be two species which he described under the names of Crepidostomum cooperi and C. canadense. In Hopkins words: "the discovery that Cooper's identification is erroneous causes one to doubt the validity of Stafford's record" of C. laureatum in other species of fish which he

reported together with his finding of <u>C</u>. <u>laureatum</u> in the trout. However, Stafford (1904) himself casts some doubt on the identity of his species from <u>Perca flavescens</u>, <u>Stizostedion vitreum</u> and <u>Necturus maculatus</u>, suggesting that it may not be the same as the form he described from the trout.

Richardson (1936), commenting on his study of some of Stafford's material from the speckled trout, concludes it is identical with <u>C</u>. <u>transmarinum</u> (Nicoll) as described by Hunninen and Hunter (1933), which has been placed by Hopkins (1934) in synonymy with <u>C</u>. <u>farionis</u>. Commenting also on Stafford's specimens from <u>Perca flavescens</u>, Richardson found them to be identical with <u>C</u>. <u>solidum</u> Van Cleave and Mueller (1932); however, Hopkins (1934), on the other hand, considers <u>C</u>. <u>solidum</u> to be identical with <u>C</u>. <u>cooperi</u>. Miller (1941a) in his study of Stafford's material concludes that Stafford's <u>C</u>. <u>laureatum</u> (= <u>Stephanophiala transmarina</u> Nicoll, 1909) is identical with <u>C</u>. <u>cooperi</u> Hopkins, 1931. The writer had the opportunity of examining this material, and came to the same conclusion as Miller.

Hopkins' basis for his claim of synonymy between <u>C</u>. <u>farionis</u> and the American form named <u>Stephano-</u> <u>phialia transmarina</u> by Nicoll in 1909 rests upon the examination of specimens of both American and European origin. However, Hopkins did not have the opportunity to study

Stafford's trout material, the description of which was used by Nicoll in the determination of <u>S</u>. <u>transmarina</u>. Therefore, in view of Miller's report, which the writer confirms, he is of the opinion that Stafford's <u>C</u>. <u>laureatum</u> from the trout should not be considered a synonym of <u>S</u>. <u>transmarina</u> Nicoll, 1909, and consequently of <u>C</u>. <u>farionis</u> (O. F. Müller, 1784); rather it should be considered as one of <u>C</u>. <u>cooperi</u> Hopkins, 1931.

The Life History of C. cooperi in Quebec

The life history of this trematode has been studied by Hopkins (1934) who also described the morphology of the various larval stages and the adult. Brown in 1927 and Crawford in 1943 reported on the life history of <u>C</u>. <u>farionis</u>, while Ameel (1937) and Henderson (1938) reported on that of <u>C</u>. <u>cornutum</u>. As shown by Brown and Hopkins, the redial and cercarial stages of species of <u>Crepidostomum</u> are to be found in Sphaeriid clams, while the encysted or metacercarial stage has been shown by Cooper (1915), Noller, Faust (Hopkins (1934)), Brown (1927), Baylis (1931), Abermathy (1937), Ameel (1937), Henderson (1938) and Crawford (1943), to occur in a variety of aquatic arthropods.

Molluscan Host Stages

In his study on the life history of C. cooperi,

Hopkins (1934) found the redial and cercarial stages of the fluke to occur in the sphaeriid <u>Musculium transversum</u> (Say) in various localities in the United States. Hopkins also recorded the infection in a single specimen of a species belonging to the genus <u>Pisidium</u>.

In the present study, the following species of clams collected in Lake Turgeon, Laurentide Park, were found to harbour the larval parasite: Pisidium subtruncatum, P. compressum, P. adbitum, P. llijeborgi and P. nitidum. In all cases the rate of infection, which was low (two to three per cent), was probably due to a seasonal variation. This is suggested by Hopkins' findings of an infection rate of sixty-seven per cent in clams collected from October to June, while the percentage of infection in the few clams he found in June and July was nil. Unfortunately, due to climatic conditions prevailing in the Laurentide Park early in the fall until late spring, it has not been possible to collect clam material during that period in order to determine whether the seasonal variation observed by Hopkins in Illinois is also to be found in Quebec.

Aquatic Arthropoda Hosts

Both Brown (1927) and Hopkins have shown in their studies on the life histories of <u>C</u>. <u>farionis</u>, <u>C</u>. <u>cooperi</u> and <u>C</u>. <u>isostomum</u> that mayfly nymphs are the second

intermediary hosts. Previously, Cooper (1915) reported his finding of encysted larvae in nymphs of ephemerids of the genus <u>Hexagenia</u>. Cooper believed these larvae to be those of <u>C</u>. <u>laureatum</u> which subsequently Hopkins considered to consist of two species, which he described under the names of <u>Crepidostomum cooperi</u> and <u>Crepidostomum canadense</u>. Cooper's record of metacercaria in <u>Hexagenia</u> nymphs is the earliest one on the developmental stages of species of <u>Crepidostomum</u>, and the only one in Canada.

In his study on the biology of C. cooperi Hopkins (1934) reported his finding the metacercaria encysted in muscle and body cavity of the nymph of the mayfly <u>Hexagenia</u> <u>limbata</u> (Guerin). He showed the rate of infection in the nymphs collected during the summer months to vary between sixty-one and ninety-five per cent. According to Hopkins there is apparently no seasonal variation in the rate of infection. During the course of the present study, the metacercarial stage of C. cooperi was found in the nymphs of the mayfly Hexagenia recurvata and in the nymph of a species of <u>Polymitarcys</u>. In the first species over eighty per cent of the nymphs examined were found to harbour the larval parasite, while the rate of infection in the second was about twenty per cent. Other aquatic arthropods, including dragonfly nymphs, damselfly naiads, Gammarus pulex, all very common in waters where fish are known to harbour C. cooperi, were examined, but were negative.

Experimental Feeding

Twelve parasite-free two year old trout were fed nymphs of <u>Hexagenia recurvata</u> and nymphs of <u>Poly-</u> <u>mitarcys</u> sp. In both cases the material fed to the fish originated in localities where infection is known to occur in the fish. In both experiments infection was successful, and Hopkins' (1934) finding that the time required by the worm to develop into sexually mature adults varied between three to four weeks, was confirmed.

<u>Crepidostomum farionis (0. F. Müller, 1784)</u> (Figure 12)

While <u>C</u>. <u>cooperi</u> is frequently encountered in the speckled trout in Quebec, <u>C</u>. <u>farionis</u> is of rare occurrence. It was found in only a few fish from streams and lakes of the Jacques Cartier and Chicoutimi drainage systems in the Laurentide Park. Fantham and Porter (1948) reported finding it in a speckled trout they had bought in Montreal. Unfortunately, these authors did not mention the locality of origin of this fish, and it is not possible to determine whether the fish was taken in Quebec or elsewhere. This is a point of importance, as at this time (1948) attempts were made to market imported speckled trout in Quebec, where its sale is forbidden by law. Under the name of <u>C</u>. <u>farionis</u>, Hopkins (1934) reports Stafford's 1904 record of <u>Crepidostomum</u> (<u>Distoma</u>) <u>laureatum</u> (Zeder) from brook trout bought at a Montreal market, presumably from Quebec. However, as shown previously in the discussion on the systematic status of Stafford's material, it proved to be <u>C. cooperi</u>.

<u>C. farionis</u> has been recorded from speckled trout in the Algonquin Park in Ontario by Bangham and Venard (1946) and MacLulich (1943b), and from the United States and Europe (Hopkins (1934)) from many species of fish in addition to salmonids. Therefore, the low incidence of this species in Quebec speckled trout is rather surprising in view of its wide geographical distribution. In view of its rare occurrence no attempts were made to study the biology of this trematode in Quebec. Its intermediary hosts in Great Britain have been shown by Brown (1927) to be species of Sphaeriid clams and mayfly nymphs, or species of <u>Gammarus</u> as shown by Baylis (1931).

CESTODA

Cestodes of the families Proteocephalidae, Amphicotylidae and Diphyllobothriidae were studied.

PROTEOCEPHALIDAE Larue, 1914, amended by Woodland, 1953. (Synonym: Icthyotaeniidae Ariola, 1899)

Members of this family are included in the new order Proteocephala created by Wardle and McLeod in 1951. Their treatise on the Zoology of Tapeworms' (1952) has the merit of presenting a clear view of the history, taxonomy, nomenclature and classification of this family. In their classification these authors have adopted the scheme put forward by Woodland between 1925-1937 (Wardle and MacLeod) and recognized only one family, Proteocephalidae, with eight subfamilies, one of which, Proteocephalinae Mola, 1929, includes the material recorded in this study.

The Proteocephalinae are well-segmented small tapeworms with holdfast provided with four cup-shaped suckers. A fifth or apical sucker functional or vestigial may be present. Parenchyma divided into cortical and medullary regions with ovary, testis, yolk glands and uterus in the latter. The yolk glands are arranged in two lateral bands. The cirro-vaginal atrium opens marginally and the uterus presents numerous lateral outgrowths. Type genus: <u>Proteocephalus</u> Weinland, 1858, <u>nec</u> de Blainville, 1828.

Genus Proteocephalus Weinland, 1858

Species Recorded in North American Salmonids

The first report of a Proteocephalus in a salmonid and, in fact, in a species of trout in North America is that of Linton who, in 1897, reported <u>Taenia</u> salvelini (= Proteocephalus salvelini Larue, 1911) (Larue (1911)) from the intestine of Cristivomer namaycush from Lake Superior. Subsequently, Larue in 1914, commenting upon his study of Linton's species, concluded that in view of the state of the material at that moment, P. salvelini must be kept on the list of inadequately described species. As no later studies have been made on the cestoda of this host in this locality, the status of P. salvelini has remained unchanged. The second report is that of Ward who in 1910 (Larue (1914)) described P. pusillus from the Sebago salmon (Salmo solar sebago) from Sebago Lake, Maine. In 1911, Larue studied specimens collected by Ward from Cristivomer namaycush from Lake Temagami, Ontario, and identified them as P. pusillus.

In 1943, MacLulich (1943a) reported and described <u>Proteocephalus parallacticus</u> from the lake trout, <u>Cristi-</u> <u>vomer namaycush</u>, the speckled trout, and the brown trout, <u>Salmo fario</u>, from lakes in the Algonquin Park, Ontario. This form was subsequently recorded from this host in the

same area by Bangham and Venard in 1946.

In 1951, Alexander found a <u>Proteocephalus</u> in the intestine of rainbow trout (<u>Salmo gairdnerii gairdnerii</u>) and in speckled trout taken from Green Lakes, Deschutes National Forest, Oregon. Alexander considered his material to belong to a new species and he described it under the name of <u>Proteocephalus salmindicola</u>. However, Dr. Ivan Pratt (1953) of the University of Oregon informed the writer that it was by mistake that one of the definitive hosts of Alexander's species was identified as <u>Salvelinus fontinalis</u> instead of <u>Salvelinus malma</u>.

The Incidence of <u>Proteocephalus</u> in the Speckled Trout

Larvae and immature adults were found in the intestine of a small number of the two hundred and ten trout taken in lakes and streams of the Chicoutimi River, Jacques Cartier River and Montmorency River drainage systems of the Laurentide Park. The immature adults (Figures 13, 14) were identified, on the basis of the presence of a fifth sucker and the characters exhibited by the vagina in its relationship to the cirrus pouch, as <u>Proteocephalus parallacticus</u> MacLulich, 1943. Very small numbers of larval stages of Proteocephalids were also found but these could not be identified as to species. As shown by Van Cleave and Mueller (1934), the presence of

such larvae is of common occurrence in fish that cannot bring them to maturity. Lyster (1940a) records such larvae in speckled trout from Lake Commandant, Montebello, Quebec.

Both <u>P. parallacticus</u> and <u>P. salmindicola</u> have been found to be abundant in both the lake trout <u>C. namaycush</u> in the Algonquin Park, Ontario, and the rainbow trout in the Green Lakes of Oregon. In the latter locality the infection in <u>Salvelinus malma</u> was extremely light and consisted of one or two worms in the fish examined by Alexander. Unfortunately, neither MacLulich (1943b) nor Bangham and Venard (1946) give any data on the intensity of the infection in the speckled trout in the Algonquin Park. However, these authors have shown that this form is more commonly encountered in the lake trout.

As stated above, Proteocephalan infection was found in very few fish in the Laurentide Park. In view of this observation and the records of MacLulich, Bangham and Venard, and Alexander on the infection of some salmonid by species of <u>Proteocephalus</u> the writer is of the opinion that the speckled trout, and even <u>Salvelinus malma</u>, are not yet established as natural hosts for species of <u>Proteophalus</u>; moreover he does not consider that their mode of feeding is conducive to building up infection. In support of the latter view one has only to consider the life history of the Proteocephalids in general and the occurrence of heavy

infection in the lake trout and the rainbow trout in Oregon. Although the life cycles of both P. parallacticus and P. salminidocola are unknown, there is no doubt that species of copepods serve as the first host for the larval parasites. On the other hand, Hunter (1929) in his study of the life history of Proteocephalus pinguis, parasitic in the pike, has shown that various fish may serve as intermediate hosts for this form, conveying it between the crustacean primary host and the digestive tract of the definitive-fish host. Hunter believes that the pike may become infected with P. pinguis either through feeding on its crustacean hosts, or through feeding on other fish which acquire the larval parasite by feeding upon the crustacean hosts. Therefore, Hunter concluded that such an alternate method would explain the general infection found in pike, for two links in the food chain rather than a single one, contribute to building up an infection. The same could apply to P. parallacticus in bringing about heavy infections in lake or rainbow trout, both voracious feeders upon many species of fish that could harbour the larval stage of this form. Light infection of the speckled trout in the Laurentide Park is to be expected as in many lakes and streams of this area the diet of the trout consists mostly of plankton and aquatic arthropods.

The Systematic Status of P. salmindicola

The main morphological character used by Alexander in differentiating his species from the form described

by MacLulich as <u>P</u>. <u>parallacticus</u> is the absence of a fifth sucker in the former. The writer had the opportunity of examining the original material used by both MacLulich and Alexander, and concluded that upon the basis of measurements alone it is impossible to differentiate these species. Moreover, examination showed the existence of a fifth sucker in Alexander's form. Accordingly, the writer feels justified in considering <u>P</u>. <u>salmonidicola</u> Alexander, 1951, a synonym of <u>P</u>. <u>parallacticus</u> MacLulich, 1943. AMPHICOTYLIDAE Nybelin, 1922, amended by

Beaver and Simer, 1940.

In the present study this family is represented by a species of the genus <u>Eubothrium</u> Nybelin, 1922, <u>Eubothrium salvelini</u> (Schrank, 1790).

Genus Eubothrium Nybelin, 1922

Eubothrium salvelini (Schrank, 1790)

(Figures 15, 16)

Morphology

Like other Eubothriids this Pseudophyllidean is characterized by its simple bothria, a body segmentation usually distinct, testes between the nerve trunks, a cirrus-pouch not unusually muscular or large, an S-shaped vagina opening anterior to the cirrus-pouch, the absence of a seminal receptacle, a dorsally situated ootype, yolk glands in two lateral zones and a uterine aperture on the ventral surface of the segment. Mensurations and morphological data of this species, found in the speckled trout in Quebec, agree with those given by Kuitunen-Ekbaum (1933) and Wardle and MacLeod (1952).

<u>E</u>. <u>salvelini</u> is a relatively small cestode measuring up to 280 mm. in length, 2.5 mm. wide. The scolex is small, measuring about 0.6 to 0.7 mm. in length

by about 0.5 mm. in width; the bothria are fairly deep and the apical disk quite distinct. The ovary is kidneyshaped, non-lobulated, median, 0.45 to 0.55 mm. wide. The yolk glands are large but few and are placed chiefly between the longitudinal muscle bundles, being interrupted by median dorsal and ventral fields. The gravid uterus is a sac with lateral outgrowths. The numerous testes are ellipsoidal to rounded in shape, measuring 0.14 to 0.18 mm. in their dorso-ventral diameter, and are laterally placed but the lateral fields of testes are connected in each proglottid by a transverse bridge. The vas deferens reaches the median line. The cirrus sac is ovoid, 0.24 mm. in length by 0.08 mm. in width and extends nearly to the poral nerve trunk.

The systematic position of <u>E</u>. <u>salvelini</u> in Canadian fish in comparison with the forms found in European fish has been discussed by Wardle (1932) and Kuitunen-Ekbaum (1933).

Distribution in North American Salmonids

<u>Eubothrium salvelini</u> has been found in both Europe and North America, and as pointed out by Richardson (1936), re-examination of much material previously identified as <u>Abothrium crassum</u> would prove it to belong to the present species. Under the name of <u>A</u>. <u>crassum</u>, Cooper in
1918 recorded the presence of cestodes in the speckled trout in Michigan, and since that time E. salvelini has been recorded from this host in other parts of the United States. For instance, Hunter and Hunter (1931) claim that in northern New York State twenty per cent of the speckled trout are infected. As shown by Wardle (1933), E. salvelini has been recorded by Cooper, Kuitunen-Ekbaum and Wardle from both western and central Canada. Wardle is of the opinion that in these areas it is probably coexistent with Salvelinus and Cristivomer. From Ontario it also has been reported in trout from lakes in the Algonquin Park by MacLulich (1943b) and by Bangham and Venard (1946). In Quebec it has been reported in speckled trout from various localities by Richardson (1936), Lyster (1940a), Fantham and Porter (1948) and by the writer (1948a).

Incidence of Infection in Speckled Trout in Quebec

In the present study, \underline{E} . <u>salvelini</u>, in mature and immature forms, was found in sixty of two hundred and ten fish from the Laurentide Park, and in this area the cestode was prevalent in fish from all seven drainage systems. In most cases the worms were located in the pyloric caeca, and to a lesser extent in the intestine; in a few cases worms were found in the stomach. <u>E. salvelini</u> was also recorded in the speckled trout from lakes in Joliette,

Laviolette, Argenteuil, Papineau and Kamouraska counties and in the Seven Island area, and found as well in fish from Knob and Star lakes in Labrador.

The intensity of infection in fish examined in this study was found to be highly variable. Some fish harbour very few worms while in others the pyloric caeca are packed with worms. There appears to be no relation between the size of fish and the presence or absence of E. salvelini. As the writer could only examine fish taken during the summer months, he is unable to state whether there is a seasonal variation in this infection of trout. However, he knows that fish taken early in May are just as liable to harbour worms, immature and mature, as those taken later in the season, and is of the opinion that during the summer months the fish continually acquire and lose infection when the worms become mature. This process becomes latent during the cold period when the fish live in the deepest part of the lakes and when the worms are prepatent.

DIPHYLLOBOTHRIIDAE Lühe, 1910

In the present study this family is represented by a species of the genus <u>Diphyllobothrium</u> Cobbold, 1858. The writer wishes to point out that the nomenclature adopted here is in conformance with existing literature pertaining to Pseudophyllidean plerocercoids in salmonids, e.g. Baylis (1945), Thomas (1947), Markowski (1949).

Genus Diphyllobothrium Cobbold, 1858

Diphyllobothriid Plerocercoids in Salmonids in North America and Incidence in Quebec

A survey of the literature shows that Diphyllobothriid plerocercoids have been reported from species of Salmonid fish from various localities in North America. Thus Hayden in 1871 (Linton (1889)), Linton (1893), Simms and Shaw (1931), Mueller (1939), Babero and Rausch (1953), Hobmaier (Haderlie (1953)) and Haderlie (1953), reported the presence of Diphyllobothriid plerocercoids in salmonids in the United States. In Canada, Wardle (1933) reported them in Salvelinus fontinalis from Ungava and Richardson (1936), Fantham and Porter (1948) from the same host in MacLulich (1943b), although he found Diphyllo-Quebec. bothriid plerocercoids in lake trout from lakes in Algonquin Park, Ontario, failed to find them in the speckled trout. Mueller (1939) believes that the Diphyllobothriid plerocercoid reported by Richardson (1936) from speckled

trout in Quebec is similar to that found in the cisco of Lake Ontario, and that it is probable that the plerocercoid in both these fish is the larval stage of a similar species of tapeworm.

In the present study the plerocercoid of a Diphyllobothriid was found in speckled trout from five of the drainage systems of the Laurentide Park, namely: Chicoutimi, Jacques Cartier, Montmorency, Ste. Anne du Nord and Malbaie. It was also found in trout from lakes in Argenteuil, Montcalm, Joliette, Laviolette and Kamouraska counties. The writer has also found it in trout from Knob Lake and Star Lake in Labrador.

The writer has reported (1948a) finding these larval cestodes in eleven of two hundred and ten fish from the Laurentide Park, i.e., an incidence of five and one-half per cent. These data are erroneous, as subsequent examination of fish in the same area showed an incidence of at least forty per cent. The reason for this discrepancy is the fact that the first data were secured following the examination of viscera which had been collected by various parties (tourists, guides, cooks, etc.) in different parts of the Laurentide Park. The viscera were usually kept at least twelve to twenty-four hours before they were examined at the field laboratory or fixed in formol. As the writer later found, the larvae

will leave the cyst within a few hours, unless the viscera are immediately fixed or kept in a cold place. Under such conditions the thin walls of the cyst collapse, rendering it almost impossible to detect previous infections. This observation is confirmed by Hickey and Harris (1947) who kept the viscera of heavily infected trout in saline at 50°C. for several days. During this period the plerocercoids remained within their cysts and showed no evidence of activity. The viscera were then placed in saline at a temperature of 15°C. and many of the plerocercoids immediately became active and left their cysts within a few minutes.

Habitat and Morphology of the Larva

These cestode larvae are found encysted on the outer surface of the stomach or the intestine. The cysts are whitish in colour, usually ovoidal in shape, and measure 3 to 4 mm. long. The larvae they contain measure up to ten to twelve millimetres in length and are quite active, this activity being marked by contraction and extension of the body. The white body is flattened with both extremities usually bluntly rounded. The bothria, 0.7 to 0.8 mm. in length, are mere slits. The number of cysts in any one fish is usually quite small, although trout harbouring fifty to sixty cysts have been

taken. That the number of cysts present can be very large is demonstrated by Fantham and Porter (1948) who reported finding fish harbouring between one to two hundred, and at an extreme, two hundred eighty six and five hundred sixty-five cysts. Richardson (1936) also reports examining a trout the stomach wall of which was so covered by cysts as to conceal the surface from view.

Pathogenicity of Diphyllobothriid Plerocercoids in Salmonids

Although in the present study the writer did not have an opportunity to observe cases where pathogenicity could be attributed to Diphyllobothriid larvae, there are several records where these larval tapeworms have been incriminated in the genesis of morbid troubles.

As far as the writer is aware, the first report of a pathogenic action attributed to Diphyllobothriid plerocercoids in salmonid fish is that of Linton who in 1891 (Linton (1893)) reported the plerocercoids of a cestode he identified as <u>Diphyllobothrium</u> (<u>Dibothrium</u>) <u>cordiceps</u> Leidy, 1871, as being deleterious to the fish harbouring them. In his discussion of the subject, Linton mentions that the trout were "infested with a parasitic worm, which is most commonly in the abdominal cavity, in cysts, but which in time escapes from the cyst and tunnels into the flesh of the host. Fish, when

thus afflicted, are found to be lacking in vitality, weak, and often positively emaciated". According to Linton, such diseased fish have a tendency to conglomerate in warm waters where they are prey to pelicans which harbour the adult parasite.

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Since Linton's report, Diphyllobothridiasis in Salmonidae has been reported: in the United States by Simms and Shaw (1931), Hobmaier (Haderlie (1953)), Mueller (1939); in Canada, by Wardle (1933), Richardson (1936), Fantham and Porter (1948); in Europe by Duguid and Sheppard (1944) and Hickey and Harris (1944) (1947) who reported epizootic outbreaks in Wales and Ireland, while infection of trout has also been reported from Northamptonshire by Gibbson and from the Shetland Islands by Peterson (Lapage (1945)).

It has been stated that infection in salmonids is often fatal (Simms and Shaw), (Hobmaier), (Duguid and Sheppard), (Hickey and Harris). Peritonitis and ascites in infected fish have been recorded by Simms and Shaw and by Wardle. According to Hickey and Harris, the majority of the infected fish (Ireland) showed a plastic peritonitis and ascites. In most cases oedomatous granulation tissue, frequently containing large extravasation of blood, was present on the stomach and intestine, often in sufficient amount to bind the stomach and pyloric caeca into a large tumour-like mass. Adhesions between the abdominal

viscera and body cavity walls were found frequently; the pallor of flesh and gill rays suggested severe anaemia. Fantham and Porter also reported similar lesions.

The Adult Forms and their Systematic Status

A survey of the literature (Markowski (1949)) indicates that Salmonid fish harbour the plerocercoids of species of Diphyllobothriid parasitic in birds. Eight species, namely: Dyphyllobothrium dendriticum (Nitzch, 1824), D. ditremum (Creplin, 1825) D. fissiceps (Creplin, 1828), D. cordiceps (Leidy, 1872), D. exile (Linton, 1892), D. canadense Cooper, 1921, D. sp. innom. Markowski, 1933 and D. oblongatum Thomas, 1946, have thus been secured from naturally or experimentally in-In addition Markowski records an additional fected hosts. two species, namely, <u>D</u>. strictum Talysin, 1932 from man and D. laruei Vergeer, 1942 obtained experimentally from a dog. Four of these species, namely, D. cordiceps, D. exile, D. laruei, D. oblongatum have been reported from the United States and one, D. canadense, from Canada.

Baylis (1945), commenting on the Diphyllobothriids known to occur in birds, inferred that their number could be reduced to two: <u>Diphyllobothrium dendri-</u> <u>ticum</u> and <u>D</u>. <u>ditremum</u>. Markowski (1949) after a comparative study of avian Diphyllobothriids and of material

secured from naturally and experimentally infected hosts, avian and mammalian, supports Baylis' point of view. Mueller (1939), in connection with his study of plerocercoid infection in ciscoes in Lake Ontario, examined herring gulls shot at Oswego, New York, and found a Diphyllobothrium which he believes to be identical with Diphyllobothrium oblongatum Thomas, 1946. Mueller finds that the worms obtained from the gulls seem to coincide for size and general characteristics with those which have been reported from gulls in Europe. Markowski has shown that in addition to morphological features differentiating these two species, D. dendriticum and D. ditremum, there appear to be differences in their host adaptation. Thus Diphyllobothrium ditremum is found only in birds of the families Phalacrocoracidae, Ardeidae, Colymbidae and Anatidae, while D. dentriticum occurs in Laridae and birds of other families (Corvidae and Pelecanidae) and experimentally also in mammals. Therefore, Markowski considers the following species to be identical with D. dendriticum: D. fissiceps, D. cordiceps, D. exile, D. canadense, D. strictus, D. laruei, D. oblongatum and D. sp. innom. Markowski, 1933. The writer is entirely in agreement with Markowski's conclusion that only two valid species are known in birds, namely: D. ditremum (Creplin, 1825) and Diphyllobothrium dendriticum (Nitzch, 1824).

Experimental Infection

During the course of the present study attempts were made to secure the adult stage of the Diphyllobothriid, the plerocercoid of which is found in the speckled trout in Quebec. Living larvae were fed to eight cats, two domestic ducklings, three Mallard ducklings, fourteen day-old chicks, three rabbits, five hamsters, two black bear cubs, two adult herring gulls, four laboratory raised young ring-bill gulls and a human volunteer (Table 2). Worms were recovered from only two of the cats and from three of the ring-bill gulls. In one of the cats the infection consisted of five immature worms, while in the other it consisted of only a scolex and a dead immature worm. The worms recovered from the gulls were also immature. In one of the gulls killed three days after initial feeding, the worms measured 2.8 centimetres, while in the other two, which were examined four and six days respectively after initial feeding, the worms measured 3 and 3.8 centimetres in length. The worms thus recovered (Fig. 17, 18) were tentatively identified as Diphyllobothrium dendriticum. The reason for this identification rests on two facts: first, Markowski's observation that specimens of <u>D</u>. <u>ditremum</u> measuring four centimeters in length are fully developed and possess gravid segments; in the present case one of the worms recovered

TABLE 2

FEEDING EXPERIMENT

Host	Larvae	Postmortem	Pagults
1030	1.90	at ter (uays)	Results
Cat (number 1)	10	6	5 immature worms
" " 3	10	13	l scolex and l dead immature worm
" (numbers 2, 4, 5, 6, 7, 8	3, 15 23, 15, 15, 25	6, 10, 15, 15, 15, 20	no worms found at autopsy
Hamsters (nos. l to 4)	10 each	5	no worms found at autopsy
Rabbits (nos. 1 to 3)	3, 5, 10	10, 15, 20	no wo rms found at autops y
Black bear cubs (1 to 2)	14, 30	one in 17 days	Weekly faecal exam. negative, no worms found at autopsy
Domestic Ducklings (1 to 2)	5, 7	15, 20	no worms found at autopsy
Mallard Ducklings (1 to 3)	10, 12, 20	25	no worms found at autopsy
Day-old Chicks (1 to 8)	4	3 - 7	no worms found at autopsy
(l to 6)	4	3	no worms found at autopsy
Adult Herring Gulls (1 to 2)	10, 20	one in 60 days	Weekly faecal exam. negative, no worms found at autopsy
Labraised ring- bill Gulls, no. 1	13	3	l worm 2.8 cm. long
no. 2	12	4	l worm 3 cm. long
no. 3	6	4	no worms found at autopsy
no. 4	15	6	l worm 3.8 cm. long
Human Volunteer on	8-10 3 occas	ions	Weekly faecal exam. negative.

from a gull is almost as long as four centimetres and is still immature; second, the fact that in the present experiment the worm attained a certain development in mammals, a possibility shown to exist in the case of \underline{D} . <u>dendriticum</u> but not in that of D. ditremum.

Discussion

Recently Babero (1952) and Babero and Rausch (1953) reported that they have succeeded in experimentally infecting cats, dogs, bears, foxes, a human volunteer and the Alaska gull (<u>Larus glaucescens</u>) by feeding these hosts plerocercoids from the rainbow trout. As reported by Babero and Rausch (1953), investigations carried on in Alaska revealed the occasional occurrence of Diphyllobothriids in piscivorous birds (gulls and bald eagles), in various canines (foxes, dogs, wolves), in bears and in man. So far Babero and Rausch have not identified the form thus observed in Alaska and, therefore, it is not yet possible to comment on its relationship to the other species of Diphyllobothriids known to infect mammals and birds.

Of the ten species of avian cestodes that have been ascribed to the genus <u>Diphyllobothrium</u>, five have been secured experimentally by feeding a variety of hosts with plerocercoids harboured by salmonids(Vergeer (1942)),

(Simms and Shaw (1931)), (Duguid and Sheppard, Unsworth, (Baylis (1945) and Lapage (1945))), (Hickey and Harris (1947)), (Thomas (1947)). It has thus been shown that in some cases avian Diphyllobothriid can adapt themselves in hosts which are physiologically unrelated (e.g. cats, rats, dogs) (Duguid and Sheppard, Unsworth, Hickey and Harris, Thomas, Vergeer). However, as shown by Baylis (1945), worms from experimentally infected mammals, as compared with those from naturally infected birds, show morphological variations which, according to this author, may be simply a result of introducing the worms into abnormal hosts.

In addition to the successful attempt of Babero and Rausch, other workers have also attempted to secure infection in man by the feeding of plerocercoids harboured by salmonids. Thus Woodbury in the United States (1935) failed to infect himself with plerocercoids from <u>Salmo</u> <u>lewisi</u>; similar results were experienced in Canada by Fantham and Porter (1948) and by the writer upon the ingestion of plerocercoids from the speckled trout. Markowski, commenting on Woodbury's result, considers it to be inconclusive in view of the fact that in some experimental hosts used by other workers, adults do not always develop. Markowski also suggests the possibility of Woodbury's having used for his experiment the plero-

cercoid of a Pseudophyllidean other than <u>D</u>. <u>cordiceps</u> (= <u>D</u>. <u>dendriticum</u>). This author also believes that, since rats, dogs, cats are susceptible to infection with <u>D</u>. <u>dendriticum</u>, it is not improbable that man may also serve as a host. This conclusion is strengthened by his belief that the form from man and described as <u>D</u>. <u>strictum</u> is identical with <u>D</u>. <u>dendriticum</u>. However, the negative results secured by Fantham and Porter and by the writer indicate that in Quebec the plerocercoid of a form identified as <u>D</u>. <u>dendriticum</u> appears not to develop in man.

As shown in Table 2, two black bear cubs were fed plerocercoids from speckled trout. Faecal and postmortem examination showed negative results. However, in view of the small number of animals used in this experiment, the writer believes this experiment inconclusive in determining whether or not the bear can harbour the adult worm. On the other hand, in view of Scott's (1932) observation that in bears in Yellowstone Park the worm (<u>D</u>. <u>cordiceps</u>) is "probably always sterile" and the scarcity of bears in Quebec in areas where infection is commonly encountered in fish, the writer is of the opinion that the bear is not an important factor in the epidemiclogy of infection in trout.

A few attempts have been made to infect cats

by feeding them plerocercoids from salmonid fish. Several British writers (Baylis (1945)), Thomas (1947), Babero and Rausch (1953) reported successful infection of cats. Unsuccessful attempts have been reported by Simms and Shaw (1931). As shown in Table 2, five immature worms were recovered from a cat killed six days after initial feeding, while in another cat killed thirteen days after feeding, only a scolex and a dead immature worm were recovered. In view of the results thus secured, the writer agrees with Baylis and with Babero and Rausch that the cat is not a suitable host, and that the possibility of the worm being expelled at an early stage is great.

Attempts have also been made to infect various species of birds by feeding plerocercoids from species of salmonids. Thus Simms and Shaw (1931) reported their unsuccessful attempts to infect ducks. Similar negative results were also experienced by the writer (Table 2). Mueller (1939) fed plerocercoids from ciscoes to newly hatched chicks in which the worms attained some growth until the rudiments of the genitalia appeared. However, the worms were rapidly thrown off and, according to Mueller, it seems that a week is the maximum time they will live in this host. In the present study attempts were made to infect day-old chicks (Table 2), but these proved unsuccessful. Mueller's results also show that

avian Diphyllobothriids can develop in birds physiologically unrelated, but it seems that the probability of the worms being expelled at an early stage is even greater than in mammals.

As shown by Markowski, several species of gulls are the definitive hosts of species of avian Diphyllobothriids. Therefore, it is logical that these hosts have been used in attempts to produce experimental infections. Experimental infections of gulls have thus been reported by Simms and Shaw (1931), Thomas (1947), Hickey and Harris (1944) and recently by Babero (1952). Thomas' studies have shown that age immunity, species immunity and body temperature are factors to be considered in the establishing of experimental infection in young gulls. Thomas has shown that young gulls can be infected during the first week after hatching, when body temperature is low, and that the tapeworms are expelled from young gulls after they reach three and a half to four weeks of age. Thomas has also shown that after the initial infection additional feeding of cysts does not increase the worm burden. As shown in Table 2, young worms were recovered from experimentally infected ring-gulls raised in the laboratory. As stated above, the worms were tentatively identified as D. dendriticum. As shown by Thomas, the age of the gull is an important factor in experimental infection, as demonstrated

by the writer's failure to infect two adult herring gulls (<u>Larus argentatus</u>).

In view of the data secured in the present study and by other workers, the writer concludes that the Diphyllobothriid plerocercoids found in speckled trout in Quebec are the larval stages of an avian form tentatively identified as <u>D</u>. <u>dendriticum</u>, and that the definitive host is a gull.

NEMATODA

The nematodes studied in the present investigation are Spirurids belonging to the families Thelaziidae Railliet, 1916, and Spiruridae Oerley, 1885.

THELAZIIDAE Railliet, 1916

Genus Rhabdochona Railliet, 1916

Systematic Status

The family Thelaziidae was created by Raillet in 1916 to include a number of genera of the superfamily Spiruroidea parasitic in mammals, birds and fish with <u>Thelazia</u> Bosc, 1819, as the type genus. In addition, Railliet transferred to this family the genera <u>Ceratospira</u> Schneider, 1866, <u>Cystidicola</u> Fischer v. Waldheim, 1798, and <u>Oxyspirura</u> Drasche, 1897, and the four new genera <u>Caleiceps, Schistorophus, Serticeps</u>, and <u>Rhabdochona</u>. Subsequently, the family Thelaziidae was amended to some extent by Baylis and Daubney (1926), by Yorke and Maplestone (1926), and by Chitwood and Wehr (1934).

In 1928, Travassos <u>et al</u> created the subfamily Rhabdochoninae which they placed in the family Spiruridae, to include the genera <u>Rhabdochona</u>, <u>Cystidicola</u>, and <u>Spinitectus</u> Fourment, 1883. These authors considered as belonging to this subfamily those forms parasitic in fish which are characterized by the presence of a bicuspid mouth with lips pronounced in some cases and rudimentary in others, and of a pharynx having the shape of an elongated chitinized tube, slightly widening in its anterior part; two unequal and dissimilar spicules and the absence of a gubernaculum; and by the presence of numerous preanal and postanal papillae.

Skrjabin (1946), in his revision of the taxonomy of the Spirurids parasitic in fish, commenting on the opinion of Travassos and his colleagues, considered that the genera Capillospirura Skrjabin, 1924, Metabronema Yorke and Maplestone, 1926, and Comephoronema Layman, 1933, should be regarded as being related to the Rhabdochoninae as defined by Travassos et al. Skrjabin admitted the validity of the subfamily Rhabdochoninae as defined by Travassos and his colleagues but was of the opinion that it should not be placed in the family Spiruridae Oerley, 1885, because of the morphological differences from Spirura Blanchard, 1849, the type genus of the family. He proposed, accordingly, the creation of a new family, Rhabdochonidae, to include those spirurids of fish characterized by a thin delicate body, a mouth opening into a funnel-shaped oral cavity, a thin, elongated pharynx, the presence of cervical papillae, a clear-out double cesophagus, unequal and dissimilar spicules, and numerous postanal papillae.

This family Skrjabin divided into three subfamilies: Rhabdochoninae, Cystidicolinae, and Spinitectinae. Members of the subfamily Rhabdochoninae of Skrjabin have a smooth cuticle and eggs without polar filaments; in this subfamily he placed <u>Rhabdochona</u> and <u>Sterliadochona</u> Skrjabin, 1946. In the Cystidicolinae the eggs are provided with polar filaments; the subfamily includes <u>Cystidicola</u>, <u>Capillospirura</u>, <u>Metabronema</u>, <u>Comephoronema</u>, and <u>Pseudocystidicola</u> Layman, 1933. In the Spinitectinae the body is spinous; it contains the single genus, <u>Spinitectus</u>.

One of the advantages of the classifications proposed by Travassos <u>et al</u> (1928) and Skrjabin (1946) is the grouping of related forms parasitic in similar hosts, that is, of Spirurid worms in fish hosts, in spite of the fact that it unites species which Chitwood and Wehr (1934) have shown to have evolved along different lines. On the other hand the writer is not willing at present to admit the validity of some of the characteristics used by Skrjabin (1946) in his differentiation of the various families, for example, polar filaments are present in some species of <u>Rhabdochona</u> and lacking in some species of <u>Cystidicola</u>. In the writer's opinion, the family Rhabdochonidae Skrjabin, 1946, should consist of only two subfamilies: Rhabdochoniae and Spinitectinae. The former would include those Spirurids of fish with a smooth cuticle,

belonging to the genera <u>Rhabdochona</u>, <u>Sterliadochona</u>, <u>Cystidicola</u>, <u>Capillospirura</u>, <u>Metabronema</u>, <u>Comephoronema</u> and <u>Pseudocystidicola</u>. The second subfamily would include only the genus <u>Spinitectus</u>.

Generic Diagnosis

The known species of this genus are, except in one case, parasitic in fish hosts, Pearse (1932) having described a species, <u>R</u>. <u>uca</u>, from the fiddler crab. The main characters used in the diagnosis of <u>Rhabdochona</u> are the morphology of the cephalic structures, the anterior part of the alimentary canal and the posterior extremity of the body, and, in the male, the number and arrangement of pre- and postanal papillae, the absence of caudal alae, the relative size of the spicules; in the female, the position of the vulva, the size of the eggs, the presence or absence of filaments on the eggs are characters of diagnostic value.

In their description of the genus <u>Rhabdochona</u> Railliet (1916), Yorke and Maplestone (1926), and Baylis and Daubney (1926) mention the presence of lips; Chitwood and Wehr (1934) have shown lips to be absent in the material studied by them. The writer's observations and those of Gendre (1921) are in accord with those of Chitwood and Wehr in this respect. One of the characteristic morphological characters of <u>Rhabdochona</u> is the

funnel-shaped prostom supported by longitudinal ribs protruding anteriorly as teeth. Chitwood and Wehr (1934) in their diagnosis of the genus give the number of these teeth at ten or twelve. However, this number may be greater as, for instance, in the form described as <u>R</u>. <u>laurentiana</u> by Lyster (1940a), <u>R</u>. <u>acuminata</u> (Molin, 1860) Gendre, 1921, and <u>R</u>. <u>kidderi</u> Pearse, 1936 (Chitwood (1938)), and others (Gustafson (1949)), where the number of teeth is fourteen; it may be smaller as in <u>R</u>. <u>paski</u> Baylis, 1926 (1928). A review of the literature shows that this valuable specific character has been overlooked in many cases, probably owing to the difficulty of preparing specimens for study. In addition to these teeth, projections have been shown to exist at the base of the prostom in some species.

In the diagnosis of the genus <u>Rhabdochona</u> the spicules of the male are given as unequal in length and dissimilar. Fujita (1927) (1928) in his description of <u>R. salvelini</u> stated that the two spicules are equal in length and thus amended the diagnosis of the genus. However, examination of Fujita's illustration of the posterior extremity of the male ((Figure 3) (1927) (1928)), shows that the spicules are definitely unequal in length with his "accessory spine" corresponding to the short spicule. Weller (1938) in his description of <u>R. ovifila</u>menta reports and illustrates the presence of a strongly

chitinized gubernaculum. The writer had the opportunity of examining the mounted specimen used by Weller in the preparation of his illustration and, in the writer's opinion, what appears to be a "gubernaculum" is an optical effect caused by a bend in the spicule. There are only two records of species of this genus in which the eggs are provided with polar filaments: <u>R. ovifilamenta</u> Weller, 1938 and <u>R. cotti</u> Gustafson, 1949.

The genus <u>Rhabdochona</u> can thus be defined: mouth without lips or pseudolabia, circumoral membrane bounding a funnel-shaped prostom supported by longitudinal thickenings projecting anteriorly as teeth, the number of which is variable, mesostom long and narrow. Oesophagus composed of two unequal parts. Male: several pairs of pre- and postanal papillae; no caudal alae, spicules unequal and dissimilar; gubernaculum absent; tail conical, pointed or provided with a chitinous process. Female: uteri opposed; vulva about middle of body; eggs elliptical, embryonated, with or without polar filaments. Parasites of aquatic vertebrates.

Type species: Dispharagus denudatus Dujardin, 1845

(Dujardin (1845)) designated by Railliet (1916).

Distribution in North America

From North America six species belonging to the genus <u>Rhabdochona</u> have been described from fish hosts. These species, the type host, and locality, are shown in Table 3.

While there is only one record of R. ovifilamenta (Weller (1938)), and two of R. laurentiana (one by Lyster (1940a) and one by the writer (1948a)), a species identified as R. cascadilla Wigdor, 1918, has been recorded by Bangham and Hunter (1939), Bangham (1944), Bangham and Venard (1946), and by Fischthal (1945a) (1945b), in a score of fish hosts from both the United States and Canada. Some of the material collected by Bangham and his associates and by Fischthal has been available for study. In addition to lending some of the material upon which published records of Rhabdochona were based, Dr. Bangham also kindly loaned to the author material which he had collected from various hosts from different localities in the State of Wisconsin, U.S.A. This material, and some collected by the writer, was studied and species identified.

From Canada, Bangham (1940) and Bangham and Venard (1946) report the presence of unidentified species of <u>Rhabdochona</u> in <u>Catostomus commersonii</u>, <u>Ameirus</u> <u>nebulosus</u>, <u>Perca flavescens</u>, <u>Lepomis gibbosus</u>, <u>Pime</u>-<u>phales promelas</u>, and <u>Coregonus clupeaformis</u>. Only

TABLE 3

Species of the Genus Rhabdochona Recorded in North American Fish Hosts

Species	Type host	Locality			
<u>R. cascadilla</u> Wigdor, 1918	<u>Semotillus atromaculatus atro-</u> <u>maculatus</u> <u>Notropia cayuga</u>	Cayuga Lake, New York State, U.S.A.			
<u>R. ovifilamenta</u> Weller, 1938	<u>Perca</u> <u>flavescens</u>	Big Stone Bay, Mackinaw Straits, Michigan State, U.S.A.			
<u>R. laurentiana</u> Lyster, 1940	<u>Catostomus</u> <u>catostomus</u>	Lake Commandant, P.Q., Canada.			
<u>R. decaturensis</u> Gustafson, 1949	Aplodinotus grunniens	Lake Decatur, Illinois, U.S.A.			
<u>R</u> . <u>cotti</u> Gustafson, 1949	<u>Cottus</u> <u>cognatus</u>	Spokane, Washington, U.S.A.			
<u>R. pellucida</u> Gustafson, 1949	Pteichocheilus oregonensis	Davis Lake, Washington, U.S.A.			
<u>R. milleri</u> Choquette, 1951	Moxostomum aureolum	Ottawa River, Pointe-au- Chene, P.Q.			

specimens from the first two of these hosts were available for study; these were found to be <u>Rhabdochona cascadilla</u>. Bangham and Hunter (1939) report also the presence of unidentified species of <u>Rhabdo-</u> chona in <u>Fundulus diaphanus menoma</u>, <u>Lepibema chrysops</u>, <u>Helioperca incisor</u>, and <u>Ambloplites rupestris</u> from Lake Erie, United States. Only some specimens from the top minnow, <u>Fundulus diaphanus menoma</u>, were available for study and these were found to belong to a genus other than <u>Rhabdochona</u>. Bangham (1944) reports the presence of a larval form of <u>Rhabdochona</u> in the digestive tract of <u>Rhinicthys atratulus meleagridis</u> from the Brule River, Wisconsin; this is probably the larval stage of <u>R</u>. <u>cascadilla</u> shown by Fischthal (1945a) (1945b) to be prevalent in this locality.

Rhabdochona cascadilla Wigdor, 1918

Redescription

For the purpose of comparison and study, attempts have been made to secure the type specimens or some other material used by Wigdor for his description of <u>R</u>. <u>cascadilla</u>. However, these attempts have been unsuccessful. More than thirty years have elapsed since Wigdor's discovery and it is rather doubtful whether this material will ever be found.

Some of Wigdor's data on the morphology of the nematode have been used rather uncritically by later workers in the determination of species. Thus, Spaul (1927), and Lyster (1940a), used the length of the oesophagus as a criterion in the differentiation of R. anguilla and R. laurentiana from R. cascadilla. However, upon study of the original description and drawing of R. cascadilla (Wigdor (1918) (fig. 1a)) and upon taking the measurements into account, one sees that Wigdor mistook the mesostom for the anterior part of the oesophagus and the anterior part of the oesophagus for the posterior portion. There is also a discrepancy between the measurements of the spicule given in Wigdor's text and those which can be made from his drawing. Wigdor gives the length of the long spicule as 40µ, with the small one being one-fifth as long. Wigdor's illustration (fig. lc), indicates that the length of the long spicule is 400µ; the value given in the text is obviously erroneous. However, the length of the spicules as given by Wigdor is used by Weller (1938) in his discussion of R_{\bullet} ovifilamenta; on the other hand, Hsu (1933) in his discussion of R. opiensis appears to be aware of this error. Gustafson (1949) in his discussion of R. cascadilla draws attention to these errors.

Another discrepancy in Wigdor's description and illustration of <u>R</u>. <u>cascadilla</u> is in the position of

the anus. The distance from the posterior extremity is given as 60µ, whereas according to the scale of the drawing (fig. 1b), it is about 130µ. Similarly, the use of this scale enables the length of the anterior portion of the cesophagus to be figured as about 200µ and to situate the nerve ring at about 115µ from the anterior end.

Wigdor's description of this species is based upon material collected from Semotilus a. atromaculatus and Notropis cayuga from Cascadilla Creek, a tributary of Lake Cayuga, New York, U.S.A. The writer is informed by Dr. D. A. Webster of the Fishery Biology Department, Cornell University, that N. cayuga was based on what is known to be Notropis heterolepis heterolepis and Notropis bifrenatus. Since all efforts to trace Wigdor's type material have failed, the writer endeavored to obtain Rhabdochona from the type hosts and exact locality of R. cascadilla. Thus, in May, 1949, through the kindness of Dr. Webster, Semotilus a. atromaculatus, Notropis cornutus cornutus, Rhinicthys a. atratulus, and Hyborhynchus notatus were secured from the type locality of R. cascadilla. Only specimens of the first three species of fish were found to harbour a Rhabdochonid nematode identified as R. cascadilla. However, a few months before (February, 1949) Gustafson submitted for publication a paper in which

he redescribes as <u>R</u>. <u>cascadilla</u> a form taken from <u>Semo-</u> <u>tilus a. atromaculatus</u> and <u>Hyborhynchus</u> <u>notatus</u> from the Embarrass River, Illinois, U.S.A.

The writer had the opportunity to examine some of Gustafson's material and to compare it with forms from fish hosts from Cascadilla Creek as well as with other material collected in the Province of Quebec, and identified as <u>R</u>. <u>cascadilla</u>. Measurements of the material taken from <u>Semotilus a</u>. <u>atromaculatus</u> are given in Table 4. Since the original type material cannot be traced and has in all probability been destroyed, the writer designates specimens from this topotypical series as neotype and neoallotype.

These are small, slender worms with a thin, smooth, unstriated cuticule. The funnel-shaped prostom is provided with fourteen teeth which protrude anteriorly and which are well seen in <u>en face</u> view (Fig. 19); internally, it has a thick lining which continues posteriorly into the vestibule. At the posterior end of the prostom, at the level of the vestibular opening, the lining is thickened with the internal surface of this thickening protruding forward as sharp points. No teeth were seen at the base of the prostom which is followed by the narrow vestibule opening in the oesophagus which is distinctly divided into a muscular anterior portion and a glandular, much longer posterior portion. The eggs

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Table of Measurements (in mm.) and of Ratios to Total Length of <u>Rhabdochona</u> <u>cascadilla</u> Wigdor, 1918, Based on Mensuration of 10 Male and 7 Female Worms from <u>Semotilus</u> <u>a</u>. <u>atromaculatus</u> from Cascadilla Creek, Cayuga Lake, N.Y.

	Male			Female				
	Min.	Max.	Average	Ratio	Min.	Max.	Average	Ratio
Total length	3.87	5.42	4.64	-	6.59	12.84	7.82	-
Oesophagus: length	1.17	1.70	1.41	1:3.3	1.60	3.09	1.92	1:4
Anterior oesophagus:						0 77	0.07	
length	0.15	0.20	0.18	1:26	0.20	0.31	0.23	1:33
Posterior oesophagus: length	1.01	1.50	1.28	1:3.6	1.43	2.78	1.68	1:4.6
Buccal cavity: length	0.014	0.016	0.015	-	0.017	0.022	0.018	
Vestibule: length	0.073	0.077	0.070	1:66	0.070	0.096	0.081	1:98
Excretory pore from								
anterior extremity	0.18	0.21	0.20	1:23	0.20	0.27	0.23	1:34
Nerve ring from ante-								
rior extremity	0.12	0.14	0.13	l:35	0.13	0.16	0.15	1:54
Tail length	0.22	0.29	0.26	1:18	0.22	0.30	0.26	1:30
Short spicule	0.10	0.11	0.11	-	-	-	-	-
Long spicule	0.39	0.44	0.42		-	-	-	-
Length of short spi-								
cule to length of								
long spicule	-	-	-	1:3.9	-	-	-	-
Vulva from anterior								
extremity	-	-	-	-	3.32	7.11	4.20	1:1.9 or 52%
Egg	-	-	-	-	33u X 17u	34 u X 18u	-	-

are 33-34µ by 17-18µ and are not filamented. <u>Female</u>: Length 6.59 to 12.85 mm.; prostom 17 to 22µ long; vestibule 70 to 96µ long; oesophagus, anterior portion 209 to 312µ and the posterior portion 1.43 to 2.785 mm. with a total length of 1.609 to 3.097 mm. The nerve ring is placed 134 to 160µ from the anterior extremity with the excretory pore behind it at 202 to 266µ from the cephalic end. The vagina is 51 to 54µ long and the vulva is equatorial in position or a little pre-equatorial. The tail is 215 to 301µ long. <u>Male</u>: Length 3.877 to 5.423 mm. long; prostom 15µ long; vestibule 73 to 74µ long; oesophagus, anterior portion

154 to 200µ and the posterior portion 1.017 to 1.503 mm. long with a total length of 1.171 to 1.703 mm. long. The nerve ring is 119 to 144µ from the anterior extremity and the excretory pore 184 to 212µ from the anterior end. The right spicule, with a reflected barb at its posterior end, is 102 to 112µ long and the left one is 385 to 443µ. The tail is 215 to 298µ long. There are seven or eight pairs of preanal papillae, subventral in position except the third pair from the cloaca which is sublateral; there are six pairs of postanal papillae subventral in position except the second pair, which is sublateral.

Examination of these data and those given in Table 4 shows that this description differs from that

given by Gustafson (1949) only in the number of preanal papillae in the male worm. However, variation in the number of preanal papillae was noted in specimens from <u>Semotilus a. atromaculatus</u> collected in the Laurentide Park; in these forms the number of preanal papillae varies between eight to twelve, while in specimens taken from the speckled trout (Figure 20) in the same locality, the number of preanal papillae ranges from nine to eleven. From <u>Catostomus commersonii commersonii</u>, forms with fourteen preanal papillae were seen. Otherwise, if all measurements and ratios are taken into account no clear distinction appears between these forms. Similar variations in the number of papillae were observed by Gnedina (1927) in a study of <u>Rhabdochona denudatus</u>.

The size of the worms is quite variable not only in specimens from various hosts but also in specimens from the same host, this variation being more evident in female worms. In his description of <u>R</u>. <u>cascadilla</u>, Gustafson (1949) mentions the presence of teeth at the base of the prostom. Examination of some of Gustafson's material as well as specimens collected by the writer, failed to show such teeth.

Nectype male and necallotype female: U.S. National

Museum Helminthological Collection, No. 47320. Type host: <u>Semotilus a. atromaculatus</u>.

Type locality: Dyer's Pond, Cascadilla Creek, a tributary of Lake Cayuga, N.Y., U.S.A.

Data from the remainder of the topotypical series are as follows: ten female and seven male specimens deposited in the Institute of Parasitology collection.

The Status of R. laurentiana Lyster, 1940

This species was first reported by Lyster (1940a) from <u>Catostomus commersonii commersonii</u>. The writer (1948a) also reported it from <u>Salvelinus fontinalis</u> from the Quebec Laurentide Park. Lyster's description was based upon the study of five males and five nonovigerous females. Lyster based the distinction between <u>R</u>. <u>laurentiana</u> and <u>R</u>. <u>cascadilla</u> on Wigdor's data on the length of the oesophagus; as shown already, this is erroneous. In his description of <u>R</u>. <u>laurentiana</u> Lyster noted the absence of a chitinous spine in the male but examination of his material shows this spine to be present. Another distinction made by Lyster was in the number of preanal papillae. However, as shown in this study this is subject to variation. Otherwise, <u>Rhabdochona laurentiana</u> is identical with <u>Rhabdochona cascadilla</u>. Therefore, as a result of this study the writer is of the opinion that <u>Rhabdo-</u> <u>chona laurentiana</u> Lyster, 1940 should be regarded as synonymous with <u>R</u>. <u>cascadilla</u> Wigdor, 1918.

Distribution of R. cascadilla in trout in Quebec

In 1948 the writer reported the presence of <u>Rhabdochona laurentiana</u> in speckled trout in Quebec. However, as shown in the present thesis, <u>R</u>. <u>laurentiana</u> Lyster should be considered as synonymous with <u>R</u>. <u>cas</u>-<u>cadilla</u> Wigdor, 1918.

In the present study <u>R</u>. <u>cascadilla</u> was found in a few trout from lakes and streams of the Chicoutimi and Jacques Cartier drainage systems of the Laurentide Park but was present in only small numbers in any one fish. It was not found in trout anywhere else in the Laurentide Park but was found, as reported by the writer (1951a), in other species of fish. The writer's record of <u>R</u>. <u>cascadilla</u> in the speckled trout is the only one in Canada although as he has shown (1951a) it has also been reported from this host in the United States. In the same paper the writer showed the wide host and geographical distribution of this species.

SPIRURIDAE Oerley, 1885

Agamospirura Henry and Sisoff, 1913

(Fig. 22 (a, b, c))

The term <u>Agamospirura</u> has been proposed to designate larval Spirurids, and it has no generic status. The present record of larval Spirurids in trout is the first one pertaining to their presence in this host.

Eleven immature Spirurids were found in trout taken during the summer of 1945 and 1946, and in material collected by personnel of the Office of Biology in 1939. One of these larvae was found encapsulated on the external surface of the stomach wall, others were found in the flesh, the ovaries, the pyloric caeca, the swim bladder, and the body cavity. They were collected from fish from lakes and rivers of the Chicoutimi, Jacques Cartier, and Ste.Anne du Nord drainage systems of the Laurentide Park.

The worms are cylindrical, brownish in colour, 25 to 34.5 mm. in length. The cuticle is thick and transversely striated. The anterior extremity when viewed laterally is conical in outline; <u>en face</u> view of the head shows that the mouth opening is elongated dorsoventrally and flanked by two trilobed pseudolabia of which the lateral lobes are the larger. There are four submedian papillae located on the base of the pseudolabia. This arrangement suggests that the dorsodorsal-laterodorsal and ventroventral-lateroventral papillae have become fused. The internal circle of papillae is apparently absent.

Cervical papillae are present, located 0.037 to 0.04 mm. from the anterior extremity. The width of the anterior extremity at the level of the cervical papillae is 0.131 to 0.149 mm. The mouth is followed by a single vestibule, 0.125 mm. in length by 8µ in diameter. The oesophagus following the pharynx is from 8 to 9 mm. long and divided into an anterior muscular portion and a posterior glandular portion that is about six times as long as the muscular one. Both parts of the oesophagus are traversed by a strong oesophageal tube. The intestine is irregular in outline and difficult to follow; it terminates in a chitinous rectum averaging 0.725 mm. in length. At the level of its junction with the intestine, conspicuous glands could be seen. The anus is terminal. Neither the excretory pore nor the nerve ring was seen in any of the specimens. In two of the immature forms there were traces of growth of the male genital rudiment.

The structure and characters of the cephalic extremity are considered to be sufficient to class these immature forms as Spiruridae and, more specifically, as being representative of the subfamily Spirurinae as defined by Chitwood and Wehr (1934).
Genus Metabronema Yorke and Maplestone, 1926

The Species of the Genus Metabronema

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In 1926, Yorke and Maplestone created the genus Metabronema (Spirurinae) to receive M. magnum (Taylor, 1925) originally described under the name of Habronema magna (Taylor (1925)). While several species have been since ascribed to this genus, its status, particularly in regard to Ascarophis Beneden, 1871, requires clarification. Van Cleave and Mueller (1934) suggest the possibility of synonymity between these two genera; Chitwood <u>et al</u> (1934) (1938) consider them to be identical. Baylis (1933) and subsequently Chitwood (1934) have pointed out the close relationship of Ascarophis and Spinitectus Fourment, 1883. The latter genus is considered by Van Cleave and Mueller (1934) to be so closely related to Cystidicoloides Skinker, 1931 (Skinker (1931b) as to almost justify synonymity. Van Cleave and Mueller (1934) have suggested that Cystidicoloides is probably a synonym of Metabronema which Baylis (1934) definitely considers to be identical with the former, a view also adopted by the writer. However, owing to the limited information available on Ascarophis the writer thinks that it is preferable, at least for the time being, to consider it as a separate entity.

Since its inception, thirteen species from fish hosts have been ascribed to this genus, to which Baylis (1934) (1935) has transferred the following species: <u>Cystidicola harwoodi</u> Chandler, 1931, <u>Cystidicola</u> (<u>Pseudocystidicola</u>) <u>skrjabini</u> Layman, 1933, <u>Cystidicola</u> fischeri Travassos <u>et al</u>, 1928, and <u>Spiroptera salvelini</u> Fujita, 1920, which has already been transferred by its author (1928) to the genus <u>Cystidicola</u>. The systematic status of the first and last of these species together with that of <u>Metabronema canadense</u> Skinker, 1931 and <u>M</u>. <u>truttae</u> Baylis, 1935 is discussed in this thesis.

As shown by the writer (1951b) species of the genus <u>Metabronema</u> have been recorded in Australia, Brazil, Canada, England, Japan, the Philippines, Russia, and the United States. At present the majority of the species reported as belonging to the genus <u>Metabronema</u> has been found in Salmonid fish. From North American fish hosts three species have been recorded: <u>M. wardlei</u> Smedley, 1934 from a species of rockfish (<u>Scorpaenichthys marmoratus</u>) from Nanaimo, B.C., <u>M. salvelini</u> (Fujita, 1920) from trout and char from both the United States and Canada (Quebec and Labrador) and <u>M. prevosti</u> Choquette, 1951 from the bullhead (<u>Ameirus nebulosus nebulosus</u>) from the Ottawa River (Quebec). <u>M. salvelini</u> was also found in material given to the writer from an unidentified species of dace from Lake Edward, Province of Quebec, and as he reported it (1951c), in the intes-

tine of a single one out of over 200 muskallunge, <u>Esox</u> <u>masquinongy</u> from the St.Lawrence watershed. The material from the muskallunge consists of only one male and one immature female; these data suggest that <u>Metabronema</u> is not normally parasitic in this host.

<u>Metabronema</u> in Trout and Char and the <u>Systematic Status of M. salvelini</u>

In 1931 Skinker described <u>M. canadense</u> from the speckled trout from Quebec and Chandler, the same year, under the name of <u>Cystidicola harwoodi</u> described a nematode from <u>Salvelinus fontinalis</u> from the Adirondacks, New York, U.S.A. The form described by Chandler was afterwards transferred by Skinker (1931b) to the genus <u>Cystidicoloides</u>. As stated above, Baylis (1934) in his study of the genera <u>Cystidicola</u>, <u>Metabronema</u> and <u>Cystidicoloides</u> concludes that the last two are synonymous. Therefore, the name of the species described by Chandler became <u>Metabronema harwoodi</u>.

Species of Metabronema have been reported from char and trout in other parts of the world. In Japan, Fujita in 1920 (Baylis (1935)) described <u>Spiroptera salvelini</u> from <u>Salvelinus malma</u> and, in 1928, reported this species from <u>Salvelinus kundscha</u>. Baylis (1935) placed this species in the genus <u>Metabronema</u>, and, in 1935, Yamaguti amplified the morphological description as <u>Meta</u>-

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bronema (Cystidicola) salvelini. Fujita in 1939 reports the finding of M. iwana in Salvelinus malma from Lake Biwa, Japan. Unfortunately, Fujita does not state whether this species is the one he described in 1928 from the same host and locality under the name of <u>Cystidicola</u> iwana; however, after a search of the literature, the writer believes that this is the case. In 1939 Fujita also reported three additonal species from the char <u>Salvelinus</u> <u>kundscha</u>, namely <u>M. kosugii</u>, <u>M. amemasu</u>, and <u>M. ishii</u>. The latter species was described originally under the name of <u>Metabronema salvelini</u> but, the name being preoccupied, it was later changed by its author (1941) to its present one.

In Great Britain, Baylis in 1935 described <u>Me-</u> <u>tabronema truttae</u> from the brown trout, <u>Salmo trutta</u>. Baylis notes the great similarity existing between this species and <u>Metabronema (Spiroptera) salvelini</u> Fujita, 1920 and <u>Metabronema canadense</u> Skinker, 1931. He even points out the fact that if all the measurements are taken into account, no clear distinction appears to exist between these forms. This is not the case with the species from the char as described by Fujita (1928) (1939), all of which possess morphological characters sufficient to distinguish them from <u>Metabronema salvelini</u>, <u>M. canadense</u>, and <u>M. truttae</u>.

Under the name of \underline{M} . <u>canadense</u> the writer (1948a)

reported his finding of this species in the speckled trout in the Laurentide Park. Lyster (1940a) reported the presence of <u>Cystidicoloides harwoodi</u> Chandler, 1931 in the speckled trout from other parts of this province. Examination of Lyster's material shows it to be identical with the form described by Skinker. This species was also found by the writer in material from a species of char (probably the Arctic Char, <u>Salvelinus arcticus</u>) from the coast of Labrador, and deposited at the Institute of Parasitology. Van Cleave and Mueller (1934) report the presence of <u>Metabronema (Cystidicoloides) harwoodi</u> Chandler in <u>Salmo fario</u> from Oneida Lake. These authors, after a study of Chandler's type material, conclude that this species is identical with <u>M. canadense</u> Skinker, this species being merely a smaller variety.

Since Skinker's description was incomplete, particularly in regard to the nature of the postanal papillae, because of the paucity of the material at her disposal, the opportunity has been taken to add to the description of this species and to attempt to clarify its relationship with the others found in char and trout. As stated by Baylis (1935) the evidence for the distinctiveness of <u>M. salvelini</u>, <u>M. canadense</u>, and <u>M. truttae</u> is very inconclusive; it rests mainly on morphological points such as the nature of the postcloacal papillae and the character of the spicules. These points were, therefore, given particular study in the specimens found by the writer.

The writer had the opportunity to study part of Miss Skinker's material but it was, unfortunately, in such a condition that it could be studied only in lateral view, and it was very difficult to obtain a clear picture of the nature of the postanal papillae in the male. This study could only confirm the observations made by Mr. J. T. Lucker (1948) of the Zoological Division of the U.S. Bureau of Animal Industry, Washington, D.C., namely, that there were five pairs of postcloacal stalked papillae; that is, one pair additional to the four shown by Skinker in her figure of the right side of the tail of the male. This additional pair is located in close proximity and median to the anterior-most of the postcloacal pair.

A ventral-dorsal view of the posterior extremity of male specimens collected by the writer, shows that there are five pairs of stalked postcloacal papillae and a sixth pair of very small papillae situated at the posterior extremity of the tail between the fifth pair (Fig. 29); it is doubtful whether they can be seen in lateral view. A sixth pair exists in <u>Metabronema truttae</u> as described and illustrated by Baylis (1935). No mention of such papillae in <u>M. salvelini</u> appears in Fujita's description (Baylis (1935)), nor Yamaguti's (1935). In the arrangement of the first two pairs of postcloacal papillae there is a similarity between the writer's material and <u>M. truttae</u> as described and illustrated by Baylis (1935); it also resembles the arrangement described by Yamaguti (1935) in respect to <u>M. salvelini</u> Fujita.

In his description of M. truttae, Baylis (1935) stated that the longer spicule is the left one while neither Fujita nor Yamaguti state whether the longer spicule is that of the right or the left side. in M. canadense, according to Skinker (1931a), the right spicule is the longer one. This could not be determined with certainty in the writer's study of Skinker's material. However, in the material at the writer's disposal the longer spicule, as in the case in M. truttae Baylis, is the left one (Fig. 29). Fujita (Baylis (1935)) defines and illustrates (Fujita (1928)) a pair of transverse processes at the tip of the longer spicule. This is not reported by Yamaguti, and Baylis was unable to observe whether or not similar processes are to be found in the form of Salmo trutta because he did not have the opportunity of studying protruded spicules.

Processes on the longer spicule could be seen in ventrodorsal view of the posterior extremity of another male in mounted specimens (Figure 29). However, the writer is of the opinion that this is not the real aspect of the extremity of the longer spicule, but that these

"transverse processes" are due to distortion brought about by pressure. In lateral view it is seen that in its distal extremity the longer spicule is curved into a trough or gutter with the inferior wall of this gutter prolonged as a sharp point, while the superior one curves inward (Fig. 29) thus accentuating the groove. Therefore, when the spicule is protruded and bent on itself, as is often the case, and slight pressure is applied, so-called "transverse processes" are produced, as shown in Fig. 29. Skinker (1931a), in her figure of the right side of the tail of the male of M. canadense, shows partly the arrangement existing at the distal extremity of the longer spicule. This aspect of the spicule was observed also in her type material. The aspect of the distal end of the spicule in lateral view is quite similar to that illustrated by Johnston and Mawson (1945) in their study of Ascarophis cooperi from Platycephalus bassensis.

In their redescription of <u>M</u>. (<u>Cystidicoloides</u>) <u>harwoodi</u>, Van Cleave and Mueller (1934) report the presence of five postanal papillae and discuss the shape of the longer spicule at its distal extremity. The writer had the opportunity of studying specimens kindly loaned by Prof. Chandler, as well as the type material of <u>M</u>. <u>har-</u> <u>woodi</u>, deposited in the Helminthological Collection of the United States National Museum. In both cases the

specimens were found to exhibit the character used as criterion in this study of species of the genus <u>Meta-</u> <u>bronema</u> in char and trout, that is, the arrangement of the postanal papillae and the shape of the left spicule.

As the result of this study, the writer believes that, on the basis of the characters of the postcloacal papillae and the spicules, the following species should be regarded as synonymous: <u>Metabronema</u> (= <u>Spiroptera</u>) <u>salvelini</u> Fujita, 1920, <u>Metabronema</u> (= <u>Cystidicola</u>) <u>harwoodi</u> Chandler, 1931, <u>Metabronema canadense</u> Skinker, 1931, and <u>Metabronema truttae</u> Baylis, 1935. By virtue of the law of priority, <u>Metabronema salvelini</u> (Fujita, 1920) must stand as the valid name of the species.

Description of Metabronema salvelini

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(Figures 21, 23, 29)

The Quebec forms are small, slender and cylindrical worms, tapering at both extremities, coarsely striated anteriorly, but the striations gradually disappear near the level of the posterior end of the cesophagus. The head measures 35-43µ in diameter. The mouth opening (Fig. 21) is dorsoventrally elongated and surrounded by two pseudolabia. Only the cephalic papillae of the external circle can be observed, and these consist of four papillae located at the base of the pseudolabia (Fig. 21). The mouth leads into a

vestibule (Fig. 23) 0.10 to 0.12 mm. long in the male and 0.12 to 0.15 mm. in the female. The oesophagus is divided into an anterior muscular portion and a posterior glandular portion, which is much the longer. The anterior portion varies between 0.65 to 0.8 mm. in the male and is about 0.8 mm. in the female; the posterior portion is 1.5 to 2.2 mm. long in the male, and 2.0 to 2.8 mm. long in the female. The total length of the oesophagus in ten males averages 2.6 mm. long, and in ten females averages 3.4 mm. long. The nerve ring is about 0.17 mm. from the anterior extremity in the male and 0.18 mm. in the female. In both sexes the excretory pore is 0.210 to 0.220 mm. from the anterior extremity. The average length of ten female specimens was 13.6 mm. and that of ten male specimens 6.9 mm.

In the female the vulva is situated about the middle of the body and the anus is about 71µ from the posterior end of the body. The thick-shelled eggs are, on the average, 41-42µ by 24-26µ.

In the male the posterior extremity (Fig. 29) is coiled and the anus is about 0.12 mm. from its tip. The caudal alae are fairly well developed and supported by papillae, of which four pairs are anterior to the cloaca and six pairs posterior to it. The postcloacal group of papillae consists of five pairs of stalked papillae and a sixth pair of very small papillae situated at the posterior extremity of the tail between the fifth pair (Fig. 29). The two spicules are unequal in length. The left spicule, with its distal extremity curved into a trough or gutter, is much longer than the right spicule, being from 0.34 to 0.38 mm. long, as compared with 0.112 to 0.138 mm.

Host: <u>Salvelinus fontinalis</u> (Mitchill) Location: Stomach Locality: Green Lake (Argenteuil County) P. Que.

Distribution of M. salvelini in Trout in Quebec

This species appears to be the most widely distributed parasitic helminth of the speckled trout in this province. It was found in one hundred and thirty-nine of two hundred and ten fish from the Laurentide Park (where infection was found in fish from all of the seven drainage systems), in fish from lakes of Joliette, Montcalm, Chicoutimi and Argenteuil counties, in nearly all of forty trout collected from lakes of the Mont Tremblant Park area and in trout from Lake Edward (Quebec County). Richardson (1936) failed to find this nematode in the trout from the latter locality. Fantham and Porter (1948) reported the presence of hair-like undetermined nematodes in the speckled trout from St. John's River; these were probably Metabronema salvelini.

As already mentioned, other reports of this nematode in trout in Quebec are those of Skinker who, in 1931, reported it as a new species under the name of <u>M. canadense</u> from trout from River Matamek, and Lyster (1940a) who reported it under the name of <u>Cystidicola harwoodi</u> from fish from Lake Commandant. These and the writer's records are the only ones pertaining to the presence of this helminth in speckled trout in Canada. However, as shown by the writer this species has been reported from several species of Salmonidae and other fish from various parts of the world.

Life History of Metabronema salvelini

The Biology of the Spirurids

The Spirurids differ in their biology from many parasitic nematodes in having no free-living stages. The biology of this group was reviewed by Seurat in 1916. In the mammalian Spirurids, the egg hatches only in the digestive tract of an invertebrate host, usually an insect, and the larva then migrates via the intestinal wall into the body cavity, where it undergoes further development and molts. In the forms parasitic in mammals, the larva encysts at this stage and, after molting a third time, the fourth larval stage is reached.

A notable exception to this rule is the larva of <u>Spirura</u> <u>gastrophila</u> (Müller, 1894) which, as shown by Seurat, continues to develop while encysted until the fourth stage is reached.

From what is known of the life history of mammalian Spirurids, one can assume that the development of the forms found in fish follow a somewhat similar pattern, that is, the infective larval stage develops in arthropods, which in turn are eaten by the fish. Although many species of Spirurids have been reported from fish, knowledge of the biology of these forms is extremely scanty. As far as the writer can ascertain, the only concrete observations on the biology of species of fish Spirurids are those of Baylis and Weller. In 1931, Baylis reported his finding in Gammarus sp., collected in Hampshire, England, the fourth larval stage of a form which he considers to be the Spirurid Cystidicola farionis. Weller, in 1938, reported infection of the crustacean Hyalella knickerbockeri upon feeding it eggs of the Spirurid Rhabdochona ovifilamenta, and described the first larval stage.

Intermediary Hosts of M. <u>salvelini</u> in Quebec and Experimental Feeding

In examining the viscera of speckled trout from Green Lake (Argenteuil County) during the summer of

1949, the writer found adult Metabronema in the stomach of some of the fish, together with partially digested arthopods. The latter were identified as remnants of nymphs of a species of mayfly, an examination of which showed them to harbour a larval stage of M. salvelini. Mayfly nymphs collected on various occasions from different streams in the Laurentide Park during the summer months of 1949, 1950 and 1952, also harboured these larvae. Contrary to what happens in the development of mammalian Spirurids, the larval stages of M. salvelini do not encyst in the arthropod host but remain free in the body cavity. In most cases only a single larva was present, although occasionally a nymph would be found harbouring two or three larvae. Larvae were recovered from mayfly nymphs which were identified through the courtesy of Dr. C. S. Wally, Division of Entomology, Science Service, Department of Agriculture, Ottawa, as larval stages of Hexagenia recurvata Morgan. Larvae were also found in the nymphs of a species of mayfly of the genus Polymitarcys. Larvae were present in about forty per cent of the Hexagenia nymphs collected from the Ste. Anne du Nord River, while only a few of the nymphs of Polymitarcys collected in Grand Lac Carré were found upon examination to harbour the parasite.

The feeding of nymphs of <u>Hexagenia</u> recurvata

from the Ste. Anne du Nord River to parasite-free trout resulted in the establishment of infection and development of adult forms in from sixty to seventy days after initial feeding.

The Third and Fourth Larval Stages of M. Salvelini

The third larval stage found in Hexagenia resembles the adult form in many respects, particularly in the morphology of its digestive tract: narrow vestibule, oesophagus divided into two portions, the first, muscular, shorter, lighter in colour, being surrounded by the nerve ring at a short distance from its anterior extremity; the second glandular portion being much longer and darker in colour. The rectum is short and provided with welldeveloped glandular structures. The excretory pore is situated at a short distance behind the nerve ring and it communicates by a short canal with a gland placed along the first portion of the oesophagus. The sexes of the third-stage larva are differentiated by the presence of a knob-like caudal appendage on the tail of the female; in the male the tail is conical and this appendage is lacking (Fig.25). In both sexes the genital tubes are in an advanced stage of development but there are no signs of spicules in the male at this stage nor any sign of a vagina and vulva in the female. Measurements of male and

female third stage larvae from <u>Hexagenia</u> recurvata are given in Table 5.

Upon gaining access to the fish host, when the arthropod harbouring it is ingested, the larva continues to grow and undergoes another molt resulting in the fourth stage (Figs. 24, 26, 27, 28). This larval stage undergoes another molt and finally the young adult emerges. During this period the larva increases in size and at the end of the fourth stage the genital organs have acquired their definitive disposition. Larvae from experimentally infected fish were examined on the ninth, twenty-first and fortieth days after initial infection. The genital organs were first noted after the fortieth day examination, although in the case of the male it is fair to presume that they would have been seen a little earlier had the larvae been examined before that time.

TABLE 5

Table of Measurements (in mm.) of Third Stage Larvae of <u>M. salvelini</u> (Fujita, 1920) Based on Mensuration of 7 Male and 10 Female Larvae from <u>Hexagenis</u> recurvata

	Male	Female
Total length	3.32 to 4.89	4.2 to 7.0
Length of Vestibule	0.067 " 0.11	0.1 " 0.128
Oesophagus: Length anterior portion	0.3 " 0.486	0.432 " 0.7 4 8
Length posterior portion	0.841 " 1.40	1.0 "1.84
Total length	1.08 " 1.84	1.45 " 2.588
Nerve ring (from anterior extremity)	0.108 " 0.150	0.147 " 0.176
Excretory pore (from anterior extremity)	0.137 " 0.195	0.185 " 0.224
Length of tail	0.064 " 0.073	0.04 "0.083

SUMMARY

Nine species of helminths parasitic in the speckled trout, <u>Salvelinus fontinalis</u> (Mitchill), in Quebec have been investigated, the literature pertaining to them reviewed, and their distribution and incidence recorded.

The author has reviewed the diagnosis of the Family Gorgoderidae Looss, 1901, and of its genus Phyllodistomum Braun, 1899, and the biology of the Gorgoderids in general. He has shown that the larval stages of Phyllodistomum lachancei Choquette, 1947, first develop in clams of the genus Pisidium and that the vigorous free-swimming corcariae attract and are eaten by damselfly naiads which act as metacercarial hosts. He found that the larva encysts in the thoracic walls of the insect shortly after ingestion and that the metacercaria excysts in the digestive tract of the fish host. He has described the larval stages and adult of Phyllodistomum lachancei. He found the infection to be prevalent in trout in some lakes of the Laurentide Park, but found that the presence of the parasites in the ureters of the trout produced only slight, if any, pathological changes.

The systematic status of Stafford's <u>Distoma</u> <u>laureatum</u> Zeder, 1800 has been clarified.

A study of the biology of <u>Crepidostomum cooperi</u>

in Quebec showed that the larval stages develop in intermediate hosts not hitherto reported for this species, namely, clams of the genus Pisidium and mayfly nymphs of the genera <u>Hexagenia</u> and <u>Polymitarcys</u>.

The distribution of the Proteocephalans and Eubothriids in North American Salmonids has been reviewed and hypotheses presented in regard to their incidence in the speckled trout. A study of the morphology of <u>Proteo-</u> <u>cephalus salmindicola</u> Alexander 1951 showed this species to be identical with <u>P. parallacticus</u> MacLulich, 1943.

The incidence of Diphyllobothriid plerocercoids in Salmonids in Quebec and other localities was discussed. In an effort to determine the adult stage, plerocercoids were fed to experimental animals and worms were recovered from cats and ring-bill gulls.

The writer has reviewed the systematic status and the generic diagnosis of the genus <u>Rhabdochona</u> Railliet, 1916 and discussed species from fish in North America. He has redescribed <u>R</u>. <u>cascadilla</u> Wigdor, 1918 and designated neotype male and neoallotype female. He has shown <u>R</u>. <u>laurentiana</u> Lyster, 1940 to be identical with <u>R</u>. <u>cascadilla</u>.

Larval Spirurids (<u>Agamospirura</u>) found in trout have been described.

The species of Metabronema from fish, and

Salmonids in particular, have been discussed. <u>M. salve-</u> <u>lini</u> has been described and its systematic status with regard to other species present in Salmonids clarified. It has been shown that the first larval stages are to be found in mayfly nymphs of the genera <u>Hexagenia</u> and <u>Poly-</u> <u>mitarcys</u>. The morphology and development of the third and fourth larval stages have been discussed.

CLAIM OF CONTRIBUTION TO KNOWLEDGE

The author claims to have contributed to the knowledge of parasitic infection of trout in Quebec as follows:

Biological Studies

He has shown that the first larval stages of <u>Phyllodistomum lachancei</u> Choquette, 1947 occur in clams of the genus <u>Pisidium</u>, that the cercarial stage encysts in damselfly naiads and that the metacercaria excysts in the digestive tract of the trout. He has contributed to the knowledge of the epidemiology and pathology of this infection in trout in Quebec.

He has found new Sphaeriid hosts harbouring the first larval stages of <u>Crepidostomum</u> Hopkins 1931, and new arthropod hosts harbouring the metacercarial stage of this trematode.

He has shown that Diphyllobothriid plerocercoids in trout in Quebec are the larval stage of an avian form.

He has, for the first time, elucidated the life cycle of a Spirurid, <u>Metrabronema salvelini</u> (Fujita, 1920), parasitic in fish, by showing that its first larval stages are to be found in mayfly nymphs (<u>Hexagenia</u> and <u>Polymitarcys</u>) and that their development is completed in the fish host.

Taxonomic and Morphological Studies

He has described the larval stages (miracidium, mother and daughter sporocysts, cercaria and metacercaria) and the adult form of <u>Phyllodistomum lachancei</u> Choquette, 1947.

He has reviewed the systematic status of Stafford's <u>Distoma laureatum</u> Zeder, 1800, and shown it to be identical with <u>Crepidostomum cooperi</u> Hopkins, 1931.

He has reviewed the systematic status and the generic diagnosis of the genus <u>Rhabdochona</u> Railliet, 1916. He has redescribed <u>Rhabdochona cascadilla</u> Wigdor, 1918, and has shown that <u>R</u>. <u>laurentiana</u> Lyster is identical with it.

He has described for the first time larval Spirurids (<u>Agamospirura</u>) parasitic in trout.

He has described <u>Metabronema salvelini</u> (Fujita, 1920) and shown it to be identical with other species reported from Salmonids in North America, Japan and England, and has described the third and fourth larval stages of this species and their development into adult form.

BIBLIO GRAPHY

- ABERNATHY, C. (1937). "Notes on <u>Crepidostomum cornutum</u> (Osborn)". Trans. Amer. Micros. Soc., 56: 206-207.
- ALEXANDER, C. G. (1951). "A new species of <u>Proteocephalus</u> (Cestoda) from Oregon trout". Jour. Parasitol. 37: 160-164.
- AMEEL, D. J. (1937). "The life history of <u>Crepidostomum</u> <u>cornutum</u> (Osborn)". Jour. Parasitol. 23: 218-220.
- ARNOLD, J. G. Jr. (1934). "Some trematodes of the common bullhead <u>Ameiurus nebulosus</u> (Le Sueur)". Trans. Amer. Micros. Soc., 53: 267-276.
- BABERO, B. (1952). "The experimental infection of Alaskan gulls (<u>Larus glaucescens</u>) with <u>Diphyllobothrium</u> sp.". Program and Abstract of 27th Annual Meeting, Amer. Soc. of Parasitol., Ithaca, New York, Sept. 8, 9, 10, 1952. Jour. Par., 38 (sup.): 23.
- BABERO, B. and RAUSCH, R. (1953). "Some observations on host relationship of <u>Diphyllobothrium</u> sp. in cats". Jour. Parasitol., 39: 226-227.
- BANCHAM, R. V. and HUNTER, G. W. III (1939). "Studies of fish parasites of Lake Erie". Distribution studies. Zoologica, New York Zoological Soc., 14: 385-448.
- BANGHAM, R. V. (1940). "Parasites of fish of Algonquin Park lakes". Trans. Amer. Fish. Soc., 70: 161-170.
- ----- (1944). "Parasites of Northern Wisconsin Fish". Trans. Wisconsin Acad. Sci., 36: 291-325.
- BANCHAM, R. V. and VENARD, C. E. (1946). "Parasites of fish of Algonquin Park lakes". Publications of the Ontario Fisheries Research Laboratories, 65: 33-46.
- BAYLIS, H. A. and DAUBNEY, R. (1926). "A synopsis of the families and genera of nematoda". British Museum (Natural History), London.
- ----- (1928). "Some parasitic worms, mainly from fishes from Lake Tanganyika". Ann. Mag. Nat. History, 1: 552-562.

- BAYLIS, H. A. (1931). "<u>Gammarus pulex</u> as an intermediate host for trout parasites". Ann. Mag. Nat. History, 7: 431-435.
- ----- (1933). "The nematode genus <u>Ascarophis</u> von Beneden". Ann. Mag. Nat. History, 11: 111-117.
- ----- (1934). "Three helminthological notes". Ann. Mag. Nat. History, 14: 115-121.
- ----- (1935). "Four new species of nematodes". Ann. Mag. Nat. History, 16: 370-382.
- ----- (1945). "On the probable identity of a cestode of the genus <u>Diphyllobothrium</u> occurring in Wales and Eire." Annals Trop. Med. and Parasit., 39: 41-45.
- BHALAREO, G. D. (1937). "Studies on the helminths of India". Trematoda. IV. Jour. Helm., 15: 97-124.
- BROWN, F. J. (1927). "On <u>Crepidostomum farionis</u> O. F. Müller (= <u>Stephanophiala laureata</u> Zeder), a distome parasite of the trout and grayling. 1. The life history". Parasitology, 19: 86-99.
- CHANDLER, A. C. (1931). "New genera and species of nematode worms". Proc. U.S. Nat. Museum, 78: 1-11.
- CHITWOOD, B. G. (1934). "Report on the collection obtained by the first Johnson-Smithsonian deep-sea expedition to the Puerto-Rican deep. Two new nematodes". Smithsonian Inst. Pubs. Misc. Col. (Publ. 3243) 91.
- ----- and WEHR, E. E. (1934). "The value of cephalic structures as characters in nematode classification, with special reference to the superfamily Spiruroidea". Z. parasitenk., 7: 273-335.
- ----- (1938). "IV. Some nematodes from the caves of Yucatan". Carnegie Inst. Wash. Pub., 491: 51-66.
- ----- and CHITWOOD, M.B. (1938). "An introduction to Nematology". Sec. 1, Part 11, M. B. Chitwood, Babylon, N.Y., 55-123.

- CHOQUETTE, L. P. E. (1947). "Phyllodistomum lachancei sp. nov., a trematode from the ureters of <u>Salve-</u> <u>linus fontinalis</u> (Mitchill), with a note on its pathogenicity". Can. Jour. Research, D, 25: 131-134.
- ----- (1948a). "Parasites of freshwater fish. IV. Internal helminths parasitic in speckled trout (<u>Salvelinus fontinalis</u> (Mitchill)) in rivers and lakes of the Laurentide Park, Quebec, Canada". Can. Jour. Research, D, 26: 204-211.
- ----- (1948b). "On the species of the genus <u>Metabro-</u><u>nema</u> Yorke and Maplestone, 1926, parasitic in trout and char". Can. Jour. Research, d, 26: 329-333.
- ----- (1951a). "On the nematode genus <u>Rhabdochona</u> Railliet, 1916 (Nematoda: Spiruroidea)". Can. Jour. Zool., 29: 1-16.
- ----- (1951b). "Description of <u>Metabronema prevosti</u> sp. nov. with a note on the genus and a list of its species and their host and geographical distribution". Can. Jour. Zool., 24: 102-108.
- ----- (1951c). "Parasites of freshwater fish. V. Parasitic helminths of the muskallunge, <u>Esox m</u>. <u>masquinongy</u> Mitchill, in the St. Lawrence watershed". Can. Jour. Zool., 29: 290-295.
- COOPER, A. R. (1915). "Trematodes from marine and freshwater fishes, including one species of ectoparasitic turbellarian". Trans. Roy. Soc. Canada, 9: 181-205.
- ----- (1918). "North American pseudophyllidean cestodes from fishes". Illinois Biol. Monograph, 4: 1-243.
- CRAWFORD, W. W. (1939). "Studies on the life history of Colorado trematodes". Jour. Par., 25 (6 sup.):26.
- ----- (1940). "The life history of a gorgoderid trematode presumably of the genus <u>Phyllodistomum</u>". Jour. Par., 26 (sup.): 38.
- ----- (1943). "Colorado trematodes studies. I. A further contribution to the life history of <u>Crepidostomum farionis</u> (Müller)". Jour. Par., 29: 379-384.

- DUGUID, J. B. and SHEPPARD, E. M. (1944). "A <u>Diphyllo-bothrium</u> epidemic in trout". Jour. Path. and Bact. 56: 73-80.
- DUJARDIN, F. (1845). "Histoire naturelle des helminthes ou vers intestinaux". Encyclopédique Roret, Paris.
- FANTHAM, H. B. and PORTER, A. (1948). "The parasitic fauna of vertebrates in certain Canadian fresh waters with some remarks on their ecology, structure and importance." Proc. Zool. Soc. London, 117: 609-649.
- FAUST, E. C. (1918). "Studies on American Stephanophialinae". Trans. Amer. Micros. Soc., 37: 183-198.
- FISCHTHAL, J. H. (1942). "Three new species of <u>Phyllo-</u> <u>distomum</u> (Trematoda: Gorgoderidae) from Michiigan fishes". Jour. Parasitol., 28: 268-274.
- ----- (1943). "A description of <u>Phyllodistomum</u> <u>etheostomae</u> Fischthal, 1942. (Trematoda: Gorgoderidae), from percid fishes". Jour. Parasitol., 29: 7-9.
- ----- (1945a). "Parasites of Northwest Wisconsin fishes". Trans. Wisconsin Acad. Sci., 37: 157-220.
- ----- (1945b). "Parasites of Brule River fishes". Trans. Wisconsin Acad. Sci., 37: 275-278.
- FUJITA, T. (1927). "On new species of nematodes from fishes of Lake Biwa". Jap. Jour. Zool., 1: 169-176.
- ----- (1928). "Further studies on nematodes from fishes of Lake Biwa". Dobuts. Zasshi, Tokyo, 40: 303-314. (Japanese with English summary).
- ----- (1939). "On the nematode parasites of the Pacific Salmon". Jour. Fac. Agr. Hokkaido Imp. Univ., 42: 239-266.
- ----- (1941). "New names for <u>Metabronema salvelini</u> Fujita and <u>Cystidicola minuta</u> Fujita". Jour. Parasitol., 27: 542.
- GENDRE, E. (1921). "Notes d'helminthologie africaine VI." Procès-Verbaux Séances de la Soc. Sci. Physiques et Naturelles de Bordeaux, 73: 148-156.

- GNEDINA, M. P. (1927). "<u>Rhabdochona</u> <u>denudata</u> (Duj. 1845) from carp fishes of the North Dvinsk Basin". Sborn. Rabot Gel'-mintol. Posv. K.I. Skrjabin, 62-65.
- GOODCHILD, G. C. (1940). "The life history of <u>Phyllodis</u>-<u>tomum solidum</u> Rankin, 1937 (Trematoda: Gorgoderidae). Jour. Par., 26 (6 sup.): 36.
- ----- (1943). "The life-history of <u>Phyllodistomum</u> <u>solidum</u> Rankin, 1937, with observations on the morphology, development, and taxonomy of the Gorgoderidae (Trematoda)." Biol. Bull. 84: 59-86.
- ----- (1945). "Additional observations on the life history of <u>Gorgodera amplicava</u> Looss, 1899". Jour. Par. 31 (sup.): 22-23.
- ----- (1948). "Additional observations on the bionomics and life history of <u>Gorgodera amplicava</u> Looss, 1899 (Trematoda: Gorgoderidae)". Jour. Parasitol., 34: 407-427.
- ----- (1950). "Establishment and pathology of gorgoderid infections in anuran kidneys". Jour. Parasitol., 36: 439-446.
- GROVES, R. E. (1945). "An ecological study of <u>Phyllodis</u>-<u>tomum solidum</u> Rankin, 1937 (Trematoda: Gorgoderidae)." Trans. Amer. Micros. Soc., 64: 112-132.
- GUSTAFSON, P. V. (1949). "Description of some species of <u>Rhabdochona</u> (nematoda: Thelaziidae)." Jour. Parasitol., 35: 534-539.
- HADERLIE, E. C. (1953). "Parasites of the fresh-water fishes of Northern California". Univ. of Calif. Pub. in Zool., 57: 1-303.
- HENDERSON, H. E. (1938). "The cercaria of <u>Crepidostomum</u> <u>cornutum</u> (Osborn)." Trans. Amer. Micros. Soc., 57: 165-172.
- HICKEY, M. D. and HARRIS, J. R. (1944). "Definitive hosts of a species of <u>Diphyllobothrium</u> causing mass infection of trout in reservoirs". Preliminary note. British Med. Jour., 2: 310.
- HICKEY, M. D. and HARRIS, J. R. (1947). "Progress of the <u>Diphyllobothrium</u> epizootic at Poulaphouca Reservoir, Co. Wicklow, Ireland". Jour. Helm., 22: 13-28.

v

- HOLL, F. J. (1929). "The Phyllodistomes of North America". Trans. Amer. Micros. Soc., 48: 48-53.
- HOPKINS, H. S. (1931). "Studies on <u>Crepidostomum</u>. II. The '<u>Crepidostomum laureatum</u>' of A. R. Cooper". Jour. Parasitol., 18: 79-90.
- ----- (1934). "The papillose Allocreadiidae. III." Biol. Monog., 12: 1-80.
- HSU, H. F. (1933). "On some species of parasitic nematodes from fishes in China". Peking Nat. Hist. Bull. 8: 147-154.
- HUNNINEN, A. V. and HUNTER, G. W. III. (1933). "On the species of <u>Crepidostomum</u> in trout". Trans. Amer. Micros. Soc., 52: 150-157.
- HUNT, J. S. (1940). "The life history of gorgoderid trematode from <u>Rana clamitans</u>." Jour. Parasitol., 36 (suppl.): 27.
- HUNTER, G. W. III (1929). "Life history studies on <u>Proteo-</u> <u>cephalus pinguis</u> La Rue". Parasitology, 21: 487-496.
- ----- and HUNTER, W. S. (1931). "Biological survey, St. Lawrence watershed. Study on fish parasites in the St. Lawrence watershed". Supp. Ann. Rep. Conservation Dept., State of New York, 1930 (1931), pp. 197-216.
- JOHNSTON, T. H. and MAWSON, P. M. (1945). "Some parasitic nematodes from South Australian marine fish". Trans. Roy. Soc. South Australia, 69: 114-117.
- JOYEUX, C. H. and BAER, J. G. (1934). "Note sur une nouvelle espèce de Trématode, (<u>Gorgoderina capsensis</u>, n. sp. ' Rev. Suisse Zool., 41: 197-201.
- JOYEUX, C. H. and BAER, J. G. (1948). "Sur une cercaire de Gorgoderina (trématodes)". Bul. Soc. Neuchâteloise des Sciences Naturelles, 71; 13-27.
- KRULL, W. H. (1933). "Notes on the life history of a frog bladder fluke". Jour. Parasitol., 20: 134.
- ----- (1935). "Studies on the life history of a frog bladder fluke, <u>Gorgodera amplicava</u> Looss, 1899". Papers of the Mich. Acad. of Sci., Arts and Letters, 20: 697-710.

- KRULL, W. H. (1936). "Additional second intermediate hosts for <u>Gorgodera amplicava</u> Looss, 1899 (Trematoda: Gorgoderidae). Proc. Helm. Soc. Wash., 3: 58.
- KUITUNEN-EKBAUM, E. (1933). "A study of the cestode genus <u>Eubothrium</u> of Nybelin in Canadian fishes". Contrib. Can. Biol. Fisheries, 8: 90-98.
- LAPAGE, G., (1945). "The broad tapeworm of man, cormorants and gulls." Nature, 155: 371.
- LA RUE, G. R. (1911). "A revision of the cestode family Proteocephalidae." Zool. Anz., 38: 473-482.
- ----- (1914). "A revision of the cestode family Proteocephalidae". Illinois Biol. Monograph, 1: 1-351.
- LEWIS, F. J. (1935). "The trematode genus <u>Phyllodistomum</u> Braun". Trans. Amer. Micros. Soc., 54: 103-117.
- LINTON, E. (1889). "A contribution to the life history of <u>Dibothrium cordiceps</u> Leidy, a parasite infesting the trout of Yellowstone Park". Bull. U.S. Comm. Fish and Fisheries, 9: 337-358.
- ----- (1893). "On fish entozoa from Yellowstone National Park". Rep. U.S. Com. Fish and Fisheries, 1889-1891: 545-564.
- LOEWEN, S. L. (1935). "A new trematode of the family Gorgoderidae". Jour. Par., 21: 194-196.
- LUCKER, J. T. (1948). Personal communication. April, 1948.
- LUTZ, A. (1926). "Trématodes et Oligochètes observés dans les canaux excréteurs du rein des Batraciens de l'Amérique Méridionale". Compt. Rend. Soc. Biol., 95: 1503.
- LYNCH, J. E. (1936). "<u>Phyllodistomum singulare</u> n. sp. a trematode from the urinary bladder of <u>Dicampto-</u> <u>don ensatus</u> (Eschscholtz), with notes on related species." Jour. Parasitol., 22: 42-47.
- LYSTER, L. L. (1940a). "Parasites of freshwater fish. II. Parasitism of speckled trout and lake trout and the fish found associated with them in Lake Commandant, Que." Can. Jour. Res., D, 18: 66-78.

- LYSTER, L. L. (1940b). "<u>Apophallus imperator</u> sp. nov., a heterophyid encysted in trout, with a contribution to its life history". Can. Jour. Res., D, 18: 106-121.
- MACLULICH, D. A. (1943a). "Proteocephalus parallacticus, a new species of tapeworm from lake trout, <u>Cristivomer namaycush</u>". Can. Jour. Res., D, 21: 145-149.
- ----- (1943b). "Parasites of trout in Algonquin Park, Ontario". Can. Jour. Res., D, 21: 405-412.
- MARKOWSKI, S. (1949). "On the species of <u>Diphyllobothrium</u> occurring in birds and their relation to man and other hosts". Jour. Helm., 23: 107-126.
- MESERVE, F. G. (1943). "<u>Phyllodistomum coatneyi</u> n. sp. a trematode from the urinary bladder of <u>Ambystoma</u> <u>maculatum</u> Shaw". Jour. Par., 29: 226-228.
- MILLER, M. J. (1940). "Black spot in fishes". Can. Jour. Comp. Med. and Vet. Sci., 4: 303-305.
- ----- (1941a). "A critical study of Stafford's report on "Trematodes of Canadian Fishes" based on his trematode collection". Can. Jour. Res., D, 19: 28-52.
- ----- (1941b). "The life history of <u>Apophallus brevis</u> Ransom, 1920". Jour. Par., 27 (suppl.): 7.
- ----- (1942). "Black spot disease of speckled trout". Rev. Can. Biol., 1: 464-471.
- MUELLER, J. F. (1939). "Parasitism and diseases in fishes of the Lake Ontario watershed". Biol. Survey (1939), 16: 211-225. State of New York Conservation Dept. Suppl. to 29th Ann. Report.
- NICOLL, W. (1909). "Studies on the classification of the digenetic trematodes". Quart. Jour. Micros. Sci., 53: 391-487.
- ----- (1924). "A reference list of the trematodes parasites of British freshwater fish". Parasitology, 16: 127-144.
- ODLAUG, T. O. (1937). "Notes on the development of <u>Gorgodera</u> <u>amplicava</u> in the final host". Biol. Bull., 72: 80-87.

- PANDE, B. P. (1937). "On the morphology and systematic position of a new bladder fluke from an Indian frog". Ann. Mag. Nat. History, 20: 250-256.
- PEARSE, A. S. (1932). "Observations on the ecology of certain fishes and crustaceans along the bank of the Malta River at Port Canning". Rec. Indian Museum, Calcutta, 34: 289-298.
- PRATT, I. (1953). Personal communication. June, 1953.
- RAILLIET, A. (1916). "La famille des Thelaziidae". Jour. Parasitol., 2: 99-105.
- RANKIN, J. S. (1937). "New helminths from North Carolina salamanders". Jour. Parasitol., 23: 29-42.
- ----- (1939). "The life cycle of the frog bladder fluke, <u>Gorgoderina attenuata</u> Stafford, 1902 (Trematoda: Gorgoderidae)." Amer. Midland Nat., 21: 276-488.
- RICHARDSON, L. R., (1936). "Observations on the parasites of the speckled trout in Lake Edward, Quebec". Trans. Amer. Fish. Soc., 66: 343-356.
- SCOTT, J. W. (1935). "On the <u>Diphyllobothrium</u> of Yellowstone Park". Jour. Parasitol., 21: 421-447.
- SEURAT, L. G. (1916). "Contribution à l'étude des formes larvaires des Nématodes parasites hétéroxènes." Bull. Scientifique de la France et de la Belgique, 49: 297-377.
- SIMMS, B. T. and SHAW, J. N. (1931). "Studies on the fishborne tapeworm <u>Dibothrium cordiceps</u> Leidy". Jour. Amer. Vet. Med. Assoc. 32 (new series): 199-205.
- SKINKER, M. S. (1931a). "Three new parasitic nematode worms". Proc. U.S. Natl. Museum, 79 (Art. 24): 1-9.
- ----- (1931b). "A redescription of <u>Cystidicola stig</u>-<u>matura</u> (Leidy), a nematode parasitic in the swim bladder of salmonoid fishes, and a description of a new nematode genus". Trans. Amer. Micros. Soc., 50: 372-379.
- SKRJABIN, K. I. (1946). "A new revision of the taxonomy of the nematodes Spirurata parasitizing fishes". Doklady Akad. Nauk SSSR, 54: 751-752.

- SPAUL, E. A. (1927). "On a new species of the nematode genus <u>Rhandochona</u>." Ann. Mag. Nat. History, 19: 636-641.
- SRIVASTAVA, H. D. (1938). "A new gorgoderid trematode from the urinary bladder of an Indian migratory fish, <u>Belone strongylura</u>." Indian Jour. Vet. Sci. and Animal Husbandry, 8: 391-393.
- STAFFORD, J. (1904). "Trematodes from Canadian fishes". Zool. Anz., 27: 481-495.
- STEELMAN, G. M. (1938). "A description of <u>Phyllodistomum</u> <u>caudatum</u> n. sp." Am. Midland Natur., 19: 613-616.
- STEEN, E. B. (1938). "Two new species of <u>Phyllodistomum</u> (Trematoda: Gorgoderidae) from Indian fishes". Am. Midland Natur., 20: 201-210.
- TAYLOR, E. L. (1925). "Notes on some nematodes in the museum of the Liverpool School of Tropical Medicine". II. Ann. Trop. Med. Parasitol., 19: 57-69.
- THOMAS, L. J. (1947). "The life cycle of <u>Diphyllobothrium</u> <u>oblongatum</u> Thomas, a tapeworm of gulls". Jour. Parasitol., 33: 107-117.
- TRAVASSOS, L., ARTIGAS, P., and PEREIRA, C. (1928). "Fauna helminthologica dos peixes de agua doce do Brasil". Arquiv. Inst. Biol. (Sao Paulo), 1: 5-68.
- VAN CLEAVE, H. J. and MUELLER, J. F. (1932). "Parasites of Oneida Lake fishes. Part 1. Description of new genera and new species". Roosevelt Wild Life Ann., 3: 1-72.
- VAN CLEAVE, H. J. and MUELLER, J. F. (1934). "Parasites of Oneida Lake fishes. Part 3. A biological and ecological survey of the worm parasites". Roosevelt Wild Life Ann. 3: 1-334.
- VERGEER, T. (1942). "Two new pseudophyllidean tapeworms of general distribution in the Great Lakes Region". Trans. Amer. Micros. Soc., 61: 373-382.
- VLADYKOV, Vadim (1942). "Etude des lacs du parc des Laurentides, 1938-1941". Office de Biologie, Départ. Chasse et Pêche, Prov. Qué., 1-66.

- WARDLE, R. A. (1932). "The cestoda of Canadian fishes. II. The Hudson Bay drainage system". Contrib. Can. Biol. Fisheries, 7: 379-403.
- ----- (1933). "The parasitic helminths of Canadian animals. I. The Cestodaria and Cestoda". Can. Jour. Res., D, 8: 317-333.
- ----- and MACLEOD, J. A. (1952). "The zoology of tapeworms". The University of Minnesota Press, Minneapolis, Minn., U.S.A.
- WELLER, T. H. (1938). "Description of <u>Rhabdochona</u> <u>ovi-</u> <u>filamenta</u> n. sp. (Nematoda: Thelaziidae) with a note on the life history". Jour. Parasitol., 24: 403-408.
- WIGDOR, M. (1918). "Two new nematodes common in some fishes of Cayuga Lake". Jour. Parasitol., 5: 29-34.
- WOODBURY, L. A. (1935). "Infectivity of the plerocercoids of <u>Diphyllobothrium cordiceps</u> (Leidy) for man". Jour. Parasitol., 21: 315.
- WU, K. (1937). "Phyllodistomes from Shanghai area. (Trematoda: Gorgoderidae)." Lingnan Sci. Jour., 16: 209-213.
- ----- (1938). "Progenesis of <u>Phyllodistomum lesteri</u> sp. nov. (Trematoda: Corgoderidae) in freshwater shrimps". Parasitology, 30: 4-19.
- YAMAGUTI, S. (1934). "Studies on the helminth fauna of Japan. Part 2. Trematodes of fishes, I." Japanese Jour. Zool., 5: 249-541.
- ----- (1935). "Studies on the helminth fauna of Japan. Part 9. Nematodes of fishes, I." Japanese Jour. Zool., 6: 338-386.
- YORKE, W. and MAPLESTONE, P.Q. (1926). "The nematode parasites of vertebrates". J. and A. Churchill, London.

EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida, unless otherwise stated.

<u>Plate I</u>

Figure 1. Configuration of the drainage systems of the Laurentide Park.

<u>Plate II</u>

- Figure 2. <u>Phyllodistomum lachancei</u> Choquette, 1947. Two of the shapes assumed by free-swimming miracidium.
- Figure 3. P. lachancei. Miracidium.

<u>Plate III</u>

- Figure 4. P. lachancei. Mother sporocyst.
- Figure 5. P. lachancei. Daughter sporocyst.
- Figure 6. Photomicrograph showing sporocysts of <u>P</u>. <u>lachancei</u> in gills.

Plate IV

- Figure 7. P. lachancei. Cercaria (low magnification).
- Figure 8. <u>P. lachancei</u>. Cercarial body showing details of anatomy.

Plate V

- Figure 9. P. lachancei. Metacercaria, encysted.
- Figure 10. P. lachancei. Adult.

<u>Plate VI</u>

- Figure 11. (a) Photomicrograph of section of <u>P</u>. <u>lachancei in situ</u> x 36.
 - (b) Photomicrograph of section of <u>P</u>.
 <u>lachancei in situ</u> x 60.
 - (c) Photomicrograph of section of normal ureter of <u>Salvelinus fontinalis</u> x 60.
 - (d) Photomicrograph of section of <u>P</u>.
 <u>lachancei in situ</u> x 60.

Plate VII

- Figure 12. <u>Crepidostomum farionis</u>. Adult.
- Figure 13. Proteocephalus parallacticus. Scolex.
- Figure 14. P. parallacticus. Adult segment.

Plate VIII

- Figure 15. <u>Eubothrium salvelini</u>. (Left) immature worm; (right) scolex of adult worm, lateral view.
- Figure 16. <u>E. salvelini</u>. Adult segment.

<u>Plate IX</u>

- Figure 17. Photomicrograph of scolex of immature <u>Diphyllobothrium</u> from experimentally infected ring-bill gull (four days old).
- Figure 18. Photomicrograph of segments of immature <u>Diphyllobothrium</u> from experimentally infected ring-bill gull (four days old).
- Figure 19. <u>Rhabdochona cascadilla</u>. <u>En face</u> view of anterior extremity.
- Figure 20. <u>R. cascadilla</u>. Posterior extremity of male from <u>S. fontinalis</u>.
- Figure 21. <u>Metabronema salvelini</u>. Sketch of cephalic pattern.

<u>Plate X</u>

- Figure 22. <u>Agamospirura</u>. (a) Lateral view of anterior extremity; (b) Posterior extremity; (c) Sketch of cephalic pattern.
- Figure 23. <u>Metabronema salvelini</u>. Anterior extremity of adult female.
- Figure 24. <u>M. salvelini</u>. Anterior extremity of 40-day old fourth stage larva.

<u>Plate X1</u>

Figure 25. <u>M. salvelini</u>. Posterior extremity of nineday old third stage larva (female, left; male, right) from experimentally infected
trout.

Figure 26. <u>M. salvelini</u>. Posterior extremity of fortyday old female fourth stage larva.

Plate XII

- Figure 27. <u>M. salvelini</u>. Posterior extremity of fortyday old male fourth stage larva.
- Figure 28. <u>M. salvelini</u>. Vulvar region of forty-day old fourth stage larva.
- Figure 29. <u>M. salvelini</u>. Adult male. Ventral view (left); lateral view (right).



PLATE I

Figure 1.



Figure 2.



Figure 3.



Figure 5.



Figure 6.







PLATE V

Figure 9.



Figure 10.





Figure 11.

PLATE VII



Figure 12.

Figure 13.



Figure 14.

PLATE VIII



Figure 15.



Figure 16.







Figure 22.



Figure 23.

Figure 24.

PLATE XI



Figure 25.



Figure 26.

