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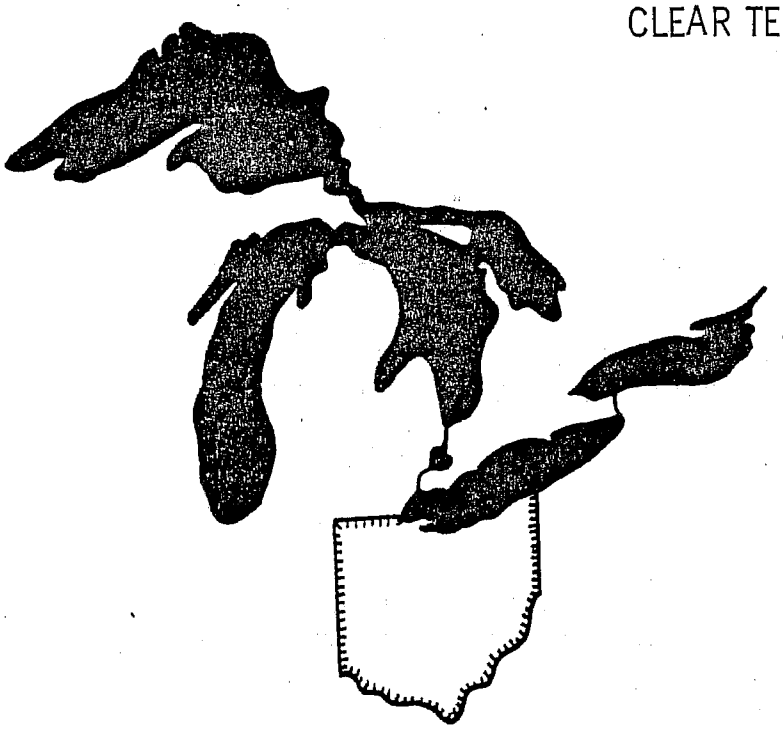

LIFE HISTORIES OF TWO PARASITIC NEMATODES WITHIN THE GENUS SPINITECTUS IN FISHES OF OHIO

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PREFACE

The following document was prepared by Reid Jilek as partial requirement for a Doctor of Philosophy Degree in the Department of Zoology of The Ohio State University. Research facilities for this dissertation were in part coordinated by the Center for Lake Erie Area Research and sponsored by the Ohio Division of Wildlife and the National Marine Fisheries Service, NOAA (Grant No. CFRD 3-298-R). Our support was provided through a Presidential Fellowship awarded by The Ohio State University. Drs. N. Wilson Britt, Ronald L. Stuckey and Charles E. Herdendorf served on the reading and examination committee; Dr. John L. Crites served as chairman of this committee and advisor.

On behalf of the Center for Lake Erie Area Research I am pleased that we are able to distribute this research report to other scientists.

Charles E. Herdendorf
Director
July 1980

TABLE OF CONTENTS

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LIST OF TABLES

LIST OF FIGURES

Figure Page

INTRODUCTION

Spinitectus carolini Holl, 1928, and Spinitectus gracilis Ward and Magath, 1916, represent two of the most common nematode parasites of freshwater fishes (Hoffman, 1967). Nevertheless, the life histories of these intestinal parasites were unknown.

The primary definitive host is the rock bass, however, smallmouth bass, largemouth bass, white bass, walleye, bluegills, sunfish, perch, catfish, sheepshead, shiners, and other Lake Erie fishes serve as final hosts for these parasites (Bangham and Hunter, 1939; Bangham, 1972).

Due to my dual interests (parasitic nematodes and fish parasites) and the awareness of the need to study these species, I began to investigate the life histories of S. carolini and S. gracilis.

Prior studies of the genus Spinitectus have been primarily of a taxonomic nature. This dissertation is the result of these investigations: 1) elucidate the life cycles of S. carolini and S. gracilis, 2) determine seasonal variation of each parasite in Lake Erie fishes, 3) histologically ascertain the pathogenicity of each parasite to their intermediate and definitive hosts, 4) ascertain intraspecific variation by scanning electron microscopy, 5) analyze and determine infected versus non-infected fish

differences by statistical techniques employing an Amdahl 470 computer.

HISTORICAL BACKGROUND

The genus Spinitectus was established by Fourment in 1883, with S. oviflagellis, from the intestines of marine mackeral fish, Meriangus vulgaris. The type species S. oviflagellis was described from female specimens only when Fourment (1883) erected the genus and its type species. Fourment (1884) provided a more detailed morphological description of the female specimens and eggs of S. oviflagellis. S. oviflagellis, S. tamari Naidenova, 1966, and S. mollis Mamaev, 1968, represent the only species of the genus possessing polar filaments on their eggs. Schuurmans-Stekhoven (1935) also recovered female specimens of S. oviflagellis from Gardus meriangus on the French coast of the North Sea.

Rahma (1964) recovered both male and female specimens of S. oviflagellis from the intestines of marine whiting, Meriangus vulgaris, collected off the west coast of Scotland. He gave a detailed morphological description of the male specimens and compared them with the males of other described species of the genus, S. cristatus and S. inermis, both of which were also found in sea-fish of Europe and America.

Rudolphi (1809) described and named some spirurroid nematodes he recovered from eels, Anguilla vulgaris, as Liorhynchus denticulatus, but these nematodes had been

previously named Goezia inermis Zeder, 1800. It was later discovered that G. inermis belonged to the genus Spinitectus, hence Goezia became a synonym of Spinitectus in part and according to Schneider, Stiles, Hassall, and Railliet in "Yorke and Maplestone's Nematode Parasites of Vertebrates, 1926", the genus Liorhynchus Rudolphi, 1873 should be abandoned. So, both Goezia and Liorhynchus become synonyms of Spinitectus in part, and both Liorhynchus denticulatus Rudolphi, 1809 and Goezia inermis Zeder, 1800 become Spinitectus inermis (Zeder, 1800). Neveu-Lemaire (1927) redescribed S. inermis in greater detail.

Linstow (1878) described a species as Filaria echinata from Alburnus lucidus and Anguilla vulgaris. This species, however, belongs to the genus Spinitectus (Railliet and Henry, 1915). According to Morishita (1926) this species S. echinatus (Linstow, 1878) appears to be synonymus with S. oviflagellis. This species has also been referred to as a larval form of S. inermis by Yorke and Maplestone, but it is now considered as a valid species of Spinitectus (Railliet and Henry, 1915).

Stewart (1914) described a single male specimen of a spiruroid nematode as Spiroptera denticulata var. minor from Wallago attu in India, but this was later established as a distinct and valid species of Spinitectus designated as S. minor (Baylis, 1936) and later redes-

cribed by Ali (1956). Railliet and Henry (1915) renamed Filaria serrata Linton, 1901 as S. cristatus.

The first described species of Spinitectus from an amphibian host was S. ranae (Morishita, 1926) from the stomachs of frogs, Rana nigromaculata, in Japan. This species was redescribed by Yamaguti (1941).

Spinitectus gigi was described from fish in Japan (Fugita, 1927) and later redecscribed by Yamaguti (1961). Yamaguti (1935) stated that they found what they believed to be a juvenile of S. gigi in a crustacean host, Caridina denticulata. Spinitectus asper was described from Prochilodus scrofa in Brazil (Travassos, Artigas, and Pereira, 1928). Travassos, Artigas and Pereira (1928) described S. yorkei from Pimelodello Lateristriga from Brazil.

Baylis (1929) described S. guntheri from an unknown fish host down 1000 meters off the coast of the southwestern part of Africa, but Campana-Rouget (1955), while working in Lakes Albert and Edwards in Africa, recovered nematodes very similar to or identical to what was described by Baylis (1929) as S. guntheri and she reassigned this species to the genus Metabronema. Since Baylis's specimens of S. guntheri are not available and can only be identified by his description, further work remains to be done either to validate this species or justify its removal and reassignment to the genus Metabronema as pro-

posed by Campana-Rouget (1955).

In India, Verma and Agarwal (1932) described S. indicus from Silurid fishes, Pseudotropius garua and Eutroplichthys vacha. In Brazil, Vaz and Pereira (1934) described S. rudolphiheringi from Pimelodella lateristraga and Salminus hilaril; and in the following year, 1935, Yamaguti described another species, S. mogurndae, from Mogurnda obscura in Japan. Moorthy (1938), added S. corti from Ophiocephalus gachua to the list from India; and this species was later redescribed by Ali (1956).

Johnston and Mawson (1940) described three species of Spinitectus from Australia: S. plectroplites (based on females only) from Percalates ombiguus; S. percalates (based on males only) from Percalates colonorum; S. bancrofti from Mogurnda adspersus. According to Khera (1954), S. percalates is a synonym of S. plectroplites.

Karve and Naik (1951) added three new species from India. These species are: S. notopteri from teleost fish Notopterus notopterus; S. mastacembeli from the stomach of Mastacembelus armatus and Notopterus notopterus; S. neilli from Barbus neilli. Khera (1954) described S. major from Mastacembelus armatus in India.

Ali (1956) added four more species from India: S. armatus from Mystus tengara; S. longipapillatus from

Rita hastata; S. singhi from Mastacembellus armatus, and S. thapari from Notopterus notopterus.

Chakravarty, Sain and Majumdar (1961) described S. bengalensis from the stomach of Notopterus notopterus in Calcutta, India. Sahay and Prasad (1965) described S. komiyai from Eutropichthyes vacha at Patna, India.

Cordero-Del-Campillo and Alvarez-Pellitero (1976) described S. gordoni from brown and rainbow trouts from northwest Spain.

The first member of the genus Spinitectus to be described from North America was S. cristatus Railliet and Henry, 1915. This species was taken from the alimentary canal of Phycis tenuis Mitchell, off Nantucket. Filaria serrata Linton 1901 nec. 1892 from the same host and locality is a synonym of this species.

Ward and Magath (1917) described S. gracilis, the first species in North America to be found in a freshwater fish. They found the parasite to be present in the alimentary tract of the black crappie (Pomoxis nigromaculatus), sheepshead (Aplodinotus grunniens), and white bass (Morone chrysops) taken from the Mississippi River at Fairport, Iowa. No figures were included in this paper, but Ward (1917) and Ward and Whipple (1918) included a drawing of the anterior end of S. gracilis.

Holl (1928) described an additional member of the

genus Spinitectus found in the freshwater fish Lepomis gibbosus and Lepomis gulosus caught near Durham, North Carolina, which he named S. carolini. Holl stated that this species was described on the basis of one female and one male specimen, although the inclusion of a range of body length implies that Holl had at least two specimens of each sex.

Mueller and Van Cleave (1932) collected specimens of Spinitectus from fishes of Oneida Lake, New York. Their studies resulted in the redescriptions of both S. gracilis and S. carolini. In addition to their own collections, Mueller and Van Cleave (1932) had available type specimens collected from the type locality (the Mississippi River at Fairport, Iowa) by Van Cleave. Their redescriptions of S. gracilis and S. carolini included the first report of the preanal and postanal papillae in male specimens, structures which had been almost completely obscured in Ward's stained material.

Holl (1932) again reported S. carolini and also a specimen of Spinitectus identified only to the generic level from Durham, North Carolina area.

Gustafson (1939) stated that mayfly larvae of the genera Hexagenia, Heptagenia, and Streptoneura readily ingest embryonated eggs of S. gracilis. He found that the advanced larval stages were found in the body cavity after three days, that specific characters appear within

eight days, and that young adults could be recovered from green sunfish, Lepomis cyanellus, as early as three days after infection with eleven day old larvae.

Christian (1969) elucidated the life cycle of S. microspinosus, which he later renamed and described as S. micracanthus (1972). Christian (1969) stated that isopods, Asellus intermedius, ingest embryonated eggs. First stage juveniles hatch in 10-12 hours, and molt to the second stage within the intestine 2-3 days later. The second stage larvae penetrates through the intestinal wall and enters the hemocoel. The second molt occurs 4-5 days later. The third stage larvae becomes encapsulated in the tissues surrounding the hemocoel. The third stage larvae is infective to the fish definitive host, Lepomis macrochirus, where it molts to the fourth stage 4-5 days post-infection of the definitive host. The last molt to the adult occurs 21-24 days later.

Keppner (1975) also did an extensive laboratory study of the development of S. micracanthus in the intermediate host, Hexagenia limbata. Keppner's results differed radically from those of Christian's for the same species of parasite. Keppner (1975) found that 12 hours post-infection with embryonated eggs, that the first stage larvae penetrated the intestinal wall and entered the hemocoel, and then entered the longitudinal

muscle bundles as they penetrated individual muscle cells. The first molt occurred 6-10 days post-infection and the second molt 19 days post-infection. The third stage then became encapsulated in the host muscle tissue and became infective to the definitive host 5 days later. The third molt to the fourth stage larvae occurred in Lepomis macrochirus 16 days post-infection. The final molt to the adult S. micracanthus occurred 25 days post-infection.

The differences in developmental times and migratory behavior of S. micracanthus may be attributed to the different first intermediate hosts utilized by Christian (isopods) and Keppner (mayfly larvae).

Overstreet (1970) described S. beaveri from the bonefish (Albula vulpes) from Biscayne Bay, Florida.

Amphibians have also been recorded as definitive hosts of Spinitectus. Holl (1928) recovered one specimen of S. carolini from the salamander, Triturus viridescens, from Durham, North Carolina. Trobridge and Hefley (1934) reported Spinitectus sp. from the bullfrog, Rana catesbeiana, from Oklahoma. While Jilek and Wolff (1978) reported recovering 12 mature adult S. gracilis from the toad, Bufo woodhouseii fowleri, from Carbondale, Illinois.

MATERIALS AND METHODS

A total of 11 species of fishes (rock bass, Ambloplites rupestris; brown bullhead, Ictalurus nebulosus; channel catfish, Ictalurus punctatus; pumpkinseed, Lepomis gibbosus; bluegill, Lepomis macrochirus; smallmouth bass, Micropterus dolomieui; largemouth bass, Micropterus salmoides; white bass, Morone chyrsops; yellow perch, Perca flavescens; white crappie, Pomoxis annularis; black crappie, Pomoxis nigromacrolatus) were collected in the western basin of Lake Erie (Fig. 1). Fish were collected by seine, fyke net, gill net, and hook and line, from June 1978 through October 1978 and from June 1979 through September 1979. Live fish were maintained for a period of not more than 2 weeks in 200 gallon aquaria filled with lake water until time of necropsy, while dead fish were necropsied immediately.

Fish were measured to the nearest mm (standard and total lengths), weighed to the nearest 0.1 gm, sexed and aged. Ageing was determined by the scale method.

Uninfected fish (bluegills, Lepomis macrochirus, in 1978; green sunfish, Lepomis cyanellus, in 1979) were obtained from the Newtown Fish Toxicology Station, Newtown, Ohio, and transported by automobile in an aerated tank to the Ohio State University laboratory on South Bass Island, Ohio. These fish were maintained in

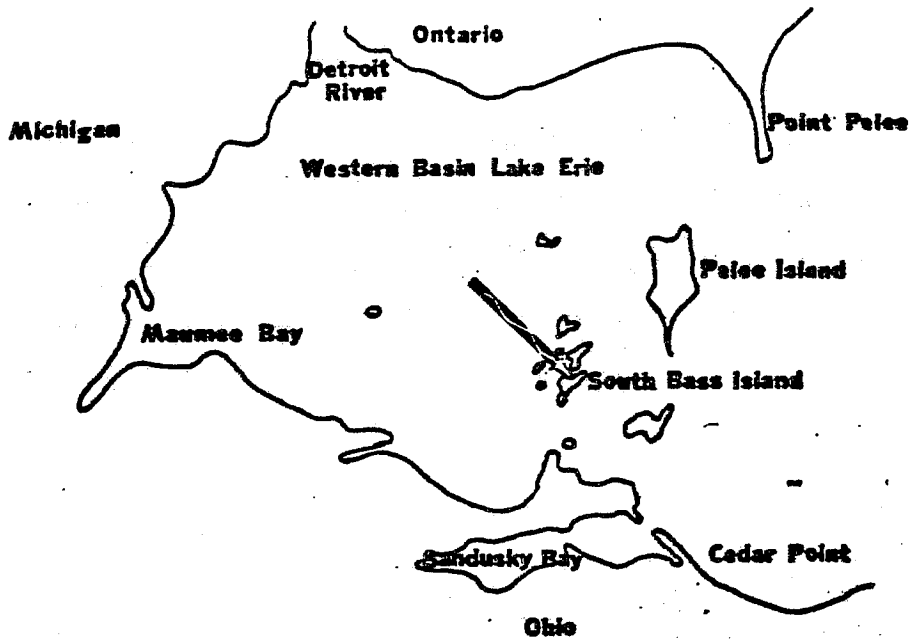

Figure 1. Arrow indicates site of collection of fish utilized in this study.

10-to 20-gallon aquaria. Water in each of these aquaria was pre-treated to eliminate bacteria, protozoa, and fungi. Approximately 25 fish were placed in each tank. All experimental fish were kept off feed for the duration of the experimental period for uniformity and control.

Experimental intermediate hosts (Table I) were collected from several areas in Ohio, however, predominantly from the Resthaven area of Northern Ohio. These animals were maintained in conditions most closely approximating their natural environments for a period of seven days, after which they were placed in fingerbowls containing double distilled water and larvated Spinitectus carolini or Spinitectus gracilis eggs. Larvated eggs had been previously acquired by mechanically rupturing mature, gravid, female Spinitectus, with the source of gravid females having been rock bass, Ambloplites rupestris. Experimental intermediate hosts were allowed to feed on larvated Spinitectus eggs for 6 hours. The seven day period previously used allowed the investigator ample time for the differentiation of natural infections to progress to a stage where they could be distinguished easily from experimental infections. All experimental infections were carried out at 20°C. Experimental infections were also carried out at ambient temperatures to ascertain temperature related differences.

Table I. List of Experimental Intermediate Hosts.

Ephemeroptera (mayflies)
1) Hexagenia limbata
2) Caenis sp.
3) Stenonema sp.
4) Heptagenia sp.
5) Baetis sp.
6) Ephemerella sp.

Odonata (dragonflies and damselflies)
1) Gomphus quadricolor
2) Pachydiplax longipennis
3) Ischnura verticalis

Plecoptera (stoneflies)
1) Neoperla clymene
2) Acroneuria sp.

Hemiptera (true bugs)
1) Belostoma fluminea
2) Notonecta undulata

Trichoptera (caddis flies)
1) Limnephilus rhombicus

Rotifera (wheel animals)
1) Keratella sp.

Diptera (flies)
1) Chironomus tentans

Isopoda (aquatic sow bugs)
1) Asellus intermedius

Amphipoda (sideswimmers)
1) Gammarus pseudolimnaeus

Hydracarina (water mites)
1) Arrenurus sp.

Ostracoda (seed shrimp)

Copepoda
1) Diaptomus sp.
2) Cyclops bicuspidatus

Cladocera (water fleas)
1) Bosmina sp.
2) Daphnia longispina

Oligochaeta (aquatic earthworms)
1) Chaetogaster sp.
2) Branchiura sowerbyi

Collembola (springtails)
1) Podura aquatica

Experimental hosts were then removed from the fingerbowls, rinsed of adhering eggs in distilled water, and returned to fingerbowls containing only distilled water. The fingerbowls were then returned to the controlled temperature chambers.

Bracketing intervals were then established to ensure complete and positive identification of stages of development and concommitant molts. Intermediate hosts were examined every 6 hours for the first 3 days and every 12 hours for the remainder of the developmental period of larval Spinitectus to the infective third stage. Some intermediate hosts were fixed in alcoholic Bouin's solution every 6 hours for the first 3 days and every 12 hours for the remainder of the developmental period. They were then prepared for histological examination to ascertain the exact position of the larvae and to determine parasite related pathology.

Uninfected bluegills and sunfish were fed intermediate hosts, previously experimentally infected and known to contain infective 3rd stage larvae of Spinitectus, or force-fed by gavage 5-10 3rd stage larvae obtained from experimentally infected intermediate hosts. These infected fish were necropsied every 6 hours for the first 3 days and every 12 hours subsequently. Sections of bluegill intestine were fixed in alcoholic Bouin's and prepared for histological examination, using standard

procedures.

Standard histological procedures utilized in this study were as follows: fixation for 24 hours in alcoholic Bouin's, dehydration in an ascending series of ethanol solutions, clearing in toulene, infiltration with paraffin at 58-59°C, embedding in paraffin, and finally sectioning at 8 um on a Spencer microtome. Staining procedures were quite variable depending on the technique utilized. Techniques used in this study were: Mallory's trichrome, hematoxylin and Eosin Y, and Lillie's allochrome.

Transfer experiments involved obtaining third and fourth stage larvae, as well as, adult Spinitectus and stomach tubing them by gavage from an infected fish to uninfected fish. Fish receiving these developmental stages of Spinitectus were then placed in aquaria for a period of two weeks, a time interval which allowed the investigator to confirm or negate the transfer of Spinitectus.

Experiments to determine the validity of S. carolini and S. gracilis as distinct species, rather than a a single species exhibiting considerable intra-specific variability, were conducted. These experiments involved infecting uninfected fish with 4th stage females of S. carolini and adult males of S. gracilis, or infections which consisted of 4th stage female S. gracilis and adult male S. carolini. Once infected the fish were maintained

for a three to four week period. This time period allowed for maturation to adult female Spinitectus and for copulation between the two species. Fertilization would provide evidence of intraspecific variation, whereas, without fertilization one could assume the variability to be interspecific.

Drawings of larval and adult stages of S. carolini and S. gracilis were prepared using a Wild tube microprojector. Photographs were taken with a Minolta XG-7 camera mounted on an AO Microstar compound microscope.

Scanning electron microscopic examinations of larval and adult stages were made with a Hitachi S 500 Stereoscan Microscope. Preparation of material for SEM was by prescribed electron micrscopic methods. These methods were as follows: worms were cleaned in Ringer's cold solution and in glacial acetic acid, fixed in gluteraldehyde with a Tris buffer or fixed in AFA, dehydrated in an ascending series of acetone solutions, dryed in a Bomar SPC 900/EX critical point dryer using CO_2 at 42°C and 13 psi for 15 minutes, attached by N. 1481 silver paint to mounting stubs, placed in a Hummer III vacuum evaporator and sputter coated with gold to a thickness of 200 A. The oscilloscope image was recorded on Polaroid PN-55 film.

All data regarding natural infections of S. carolini and S. gracilis was punched on computer cards.

THE LIFE HISTORY OF SPINITECTUS CAROLINI

Adult Spinitectus carolini occurred in the pyloric caeca and intestinal tracts of fishes. Females were oviparous. Gravid females released larvated eggs which were subsequently voided from the digestive tract with the feces. The eggs then settled to the bottom.

The stages of development (Plate I, Figs. 1-4) occurred within the uterus of the female and yielded a first stage larva (Plate I, Fig. 5).

First stage larva (Plate I, Fig. 5)

First stage larvae slender 285 to 297 (Mean 291) long, 26 to 31 (27) wide. Anterior end rounded, has pointed egg tooth. Stoma indistinct. Nerve ring 44 to 57 (45) from anterior end. Excretory pore 72 to 76 (75) from anterior end. Esophagus thin walled, 135 to 150 (142) long. Intestine indistinct, four rectal cells. Genital primordium composed of 6 cells, 245 to 250 (249) from anterior end. No mucrones present.

Determination of the intermediate host and larval development.

Intermediate hosts were infected as prescribed in the materials and methods. Spinitectus carolini infections occurred in mayfly naiads (Baetis, Caenis, Ephemerella, Heptagenea, Hexagenia, and Stenonema), dragonfly nymphs (Gomphus and Pachydiplax), stonefly

Table II. Experimental Determination of the Intermediate Host of *Spinitectus carolini*, and Natural Infections.

Potential Host	Natural Infection	Experimental Infection
Hexagenia	+	+
Caenis	-	+
Stenonema	-	+
Heptagenea	+	+
Baetis	-	+
Ephemerella	+	+
Gomphus	-	+
Pachydiplax	-	+
Ischnura	-	+
Neoperla	+	-
Acroneuria	-	+
Belostoma	-	-
Notonecta	-	-
Limnephilus	-	-
Keratella	-	-
Chironomus	+	-
Asellus	-	-
Gammurus	-	-
Arrenurus	-	-
Ostracods	-	-
Diaptomus	-	-
Cyclops	-	-
Bosmina	-	-
Daphnia	-	-
Chaetogaster	-	-
Branchiura	-	-
Podura	-	-

larvae (Ischnura and Neoperla), and midge larvae (Chironomus). Information pertaining to natural and experimental infections of intermediate hosts is presented in Table II.

First stage larva hatched from the eggs within the midgut of the insect intermediate host. The larvae penetrated through the midgut wall within the first 6 hours and entered the insects hemocoel. The first molt began 18 hours post-infection within the hemocoel and terminated by hour 36 to yield a second stage larva. A considerable degree of development occurred just prior to and just after the first molt. The stoma became oval shaped. The buccal capsule formed. The esophagus became thicker walled and thereafter differentiated into two parts, a muscular and a glandular portion. The intestinal lumen appeared. The genital primordium exhibited an increase in cell number. Body spines appeared.

Second stage larva (Plate I, Figs. 6, 7, & 8)

Second stage larvae slender 386 to 403 (398) long, 28 to 32 (30) wide. Nerve ring 84 to 89 (87) from anterior end. Excretory pore 96 to 102 (98) from anterior end. Thick walled esophagus composed of two parts: glandular 101 to 105 (104) long and muscular 64 to 76 (73) long. Intestinal lumen present, has distinct cell lining. Genital primordium 301 to 320 (309) from anterior end. Four rectal cells visible surrounding anus. No mucrones pres-

Figure 2. First stage larva of S. carolini in midgut wall of mayfly naiad, Hexagenia limbata. 100x.

ent. Spines on anterior one-fourth of body.

The second stage larvae grew and developed within the hemocoel for 5 days, after which they penetrated the abdominal muscles of the insect intermediate hosts. The larvae became encapsulated by host tissues. The second molt to the third stage larva occurred 8 days post-infection within the encapsulation in the insects abdominal muscle.

Third stage larva (Plate I, Fig. 9)

Third stage larvae slender 1310 to 1440 (1380) long, 35 to 38 (36) wide. Anterior end rounded. Oral aperature terminal, two lateral pseudolips, two medial amphids on pseudolips. Four small papillae, two subventral, two subdorsal. Nerve ring 102 to 111 (107) from anterior end. Excretory pore 134 to 148 (142) from anterior end. Esophagus 800 to 825 (810) long, muscular region 174 to 177 (175) long, glandular region 626 to 648 (635) long. Genital primordium 750 to 834 (800) from anterior end, increased substantially in cell number.

The third stage larvae were as the second stage larvae, encapsulated in the abdominal muscle tissues of the insect intermediate hosts. The third stage larvae were characterized by an increased size and development of the genital primordium. The capsules became impregnated with melanin (Jilek and Crites, 1980a) as they persisted in the abdominal muscles and were seen easily through the body wall. The larvae remained in a rela-

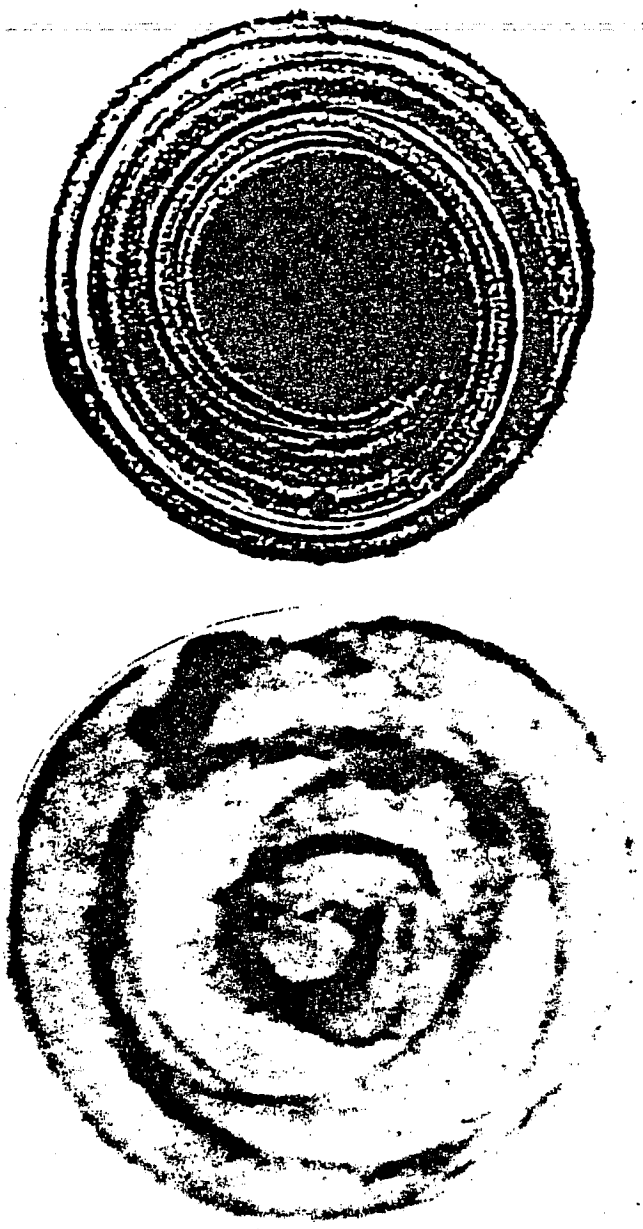

Figure 3. Top--encapsulated third stage larva of S. carolini, 10 days post-infection. 40X. Bottom--encapsulated third stage larva of S. carolini, 21 days post-infection. Note thicker capsule (contains melanin deposits). 40X.

tively quiescent state. The number and shape of the spines increased. Deirids were seen between the first and second rows of spines, along the lateral lines. Mucrones were present, and served as a means of differentiating females from males, as mucrones were present on only female S. carolini. The stoma assumed the characteristic appearance of the adult.

Determination of the definitive host

The definitive hosts were infected with third stage larvae of S. carolini as prescribed in the materials and methods section. Third stage larvae were infective to the definitive host 14 days post-infection of the intermedaite host.

Fourth stage larva

Long oral vestibule. Two lateral pseudolips, each with single amphid containing double pore. Four papillae, two subventral, two subdorsal. Deirids present between first and second rows of spines. No spines over lateral lines, point at which rows of spines break. Spines occur over entire body surface, fewer and more dispersed from anterior to posterior.

Male: Fourth stage larvae slender 2270 to 2575 (2457) long, 66 to 94 (80) wide. Nerve ring 111 to 129 (122) from anterior end. Excretory pore 157 to 180 (169) from anterior end. Esophagus 930 to 1181 (992) long, muscular region 135 to 172 (150) long, glandular region 795 to 1109

(942) long. Narrow alae present posteriorly. Four pairs of pedunculate preanal and five pairs of pedunculate postanal papillae. Two spicules, right short, left long. No gubernaculum. Testis single reflexed, extends anteriorly to level of separation of muscular and glandular esophagus. Parallel cuticular cleats present around anal region.

Female: Fourth stage larvae slender 2667 to 3280 (2978) long, 75 to 99 (88) wide. Nerve ring 125 to 139 (132) from anterior end. Esophagus 1257 to 1628 (1495) long, muscular region 137 to 225 (202) long, glandular region 1120 to 1403 (1293) long. Vulva in middle of body. ovejector well developed. Vagina approaches ovejector from posterior. Uterus amphidelphic. Mucron present.

After being freed from the capsule, within the fishes intestine, the infective third stage larva either subsisted in the intestinal lumen, penetrated the intestinal wall and subsisted in the submucosa, or penetrated through the entire intestinal wall and became encapsulated by host tissues in the mesenteries surrounding the intestine. The third molt to the fourth stage larva occurred 2 days post-infection of the fish definitive host in each of the three distinct microhabitats (intestinal lumen, submucosa, or mesenteric encapsulation). The fourth stage larva underwent rapid growth and differentiation of the gonadal tissues. Male structures such as: spicules, single reflexed testis, and caudal papillae

were visible. Female development included: formation of the vulva, uterus, ovejector and vagina.

Adult (Plate II, Figs. 10, 11, 12, 13, & 14)

Long oral vestibule, straight and thin walled. Vestibule joins muscular esophagus at level of second transverse row of spines. Diameter of muscular esophagus is one-half diameter of glandular esophagus. Nerve ring is located between fourth and fifth rows of spines. Excretory pore situated between eight and ninth rows of spines. Spines are very long and sharp, with 15 to 20 per row. Discontinuity in rows of spines over the lateral lines, more marked, further posterior. Spines occur on projections of cuticula, decrease in number, size from anterior to posterior. Head bears 4 double papillae, two lateral amphids, situated laterally on pseudolips. Each amphid bears two pore like openings. Deirids lateral, between 1st and 2nd rows of spines.

Male: Adults are slender 2783 to 3450 (3309) long, 87 to 106 (98) wide. Nerve ring 133 to 151 (145) from anterior end. Excretory pore 187 to 221 (202) from anterior end. Muscular esophagus 172 to 266 (186) long, glandular esophagus 1201 to 1497 (1403) long. Narrow alae present. Four pedunculate preanal and five pedunculate postanal papillae on either side of tail. Right spicule short, tip provided with large ventral barb, distal end pointed, proximally expanded. Left spicule long, slender, evenly curved

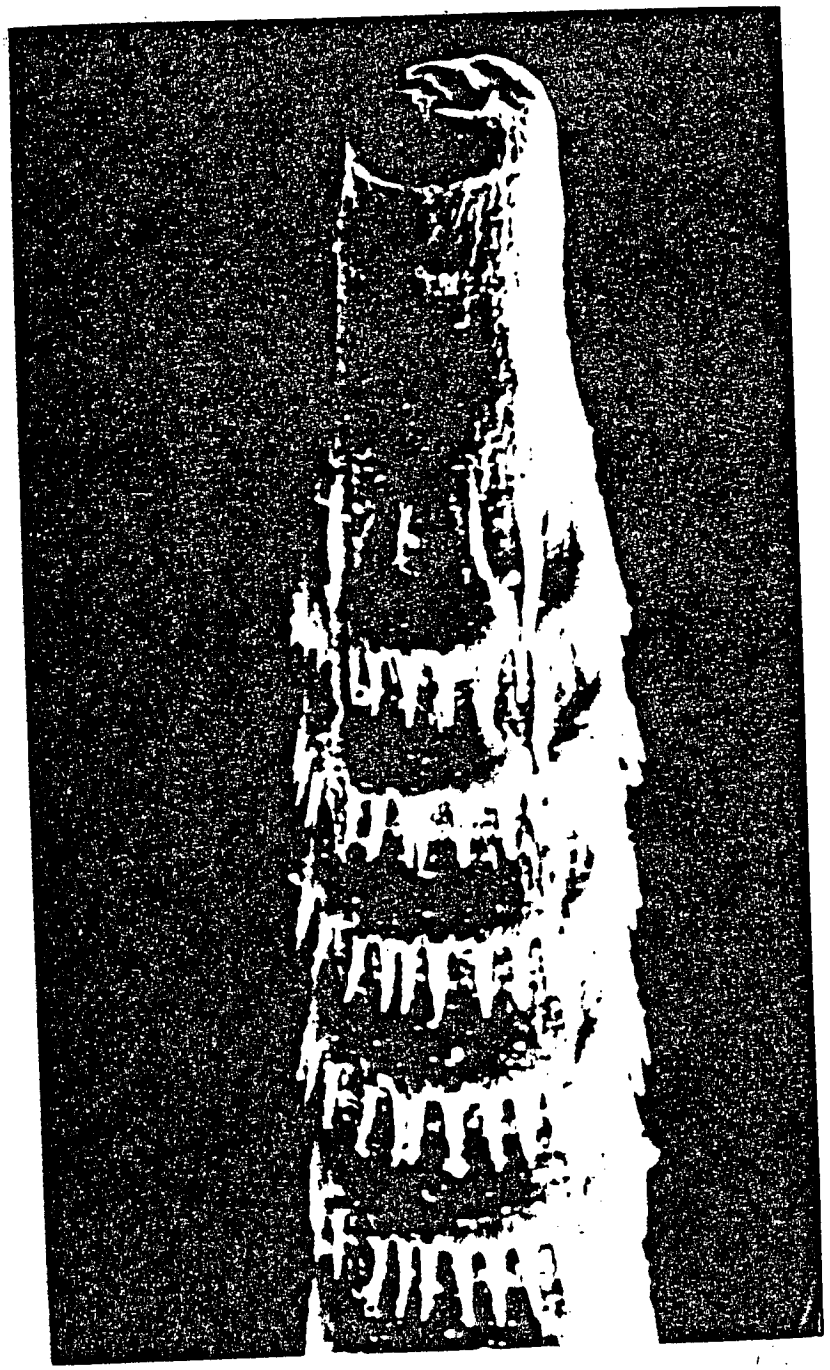

Figure 4. Anterior end of E. carolini as seen by SEM. (30um ——————)

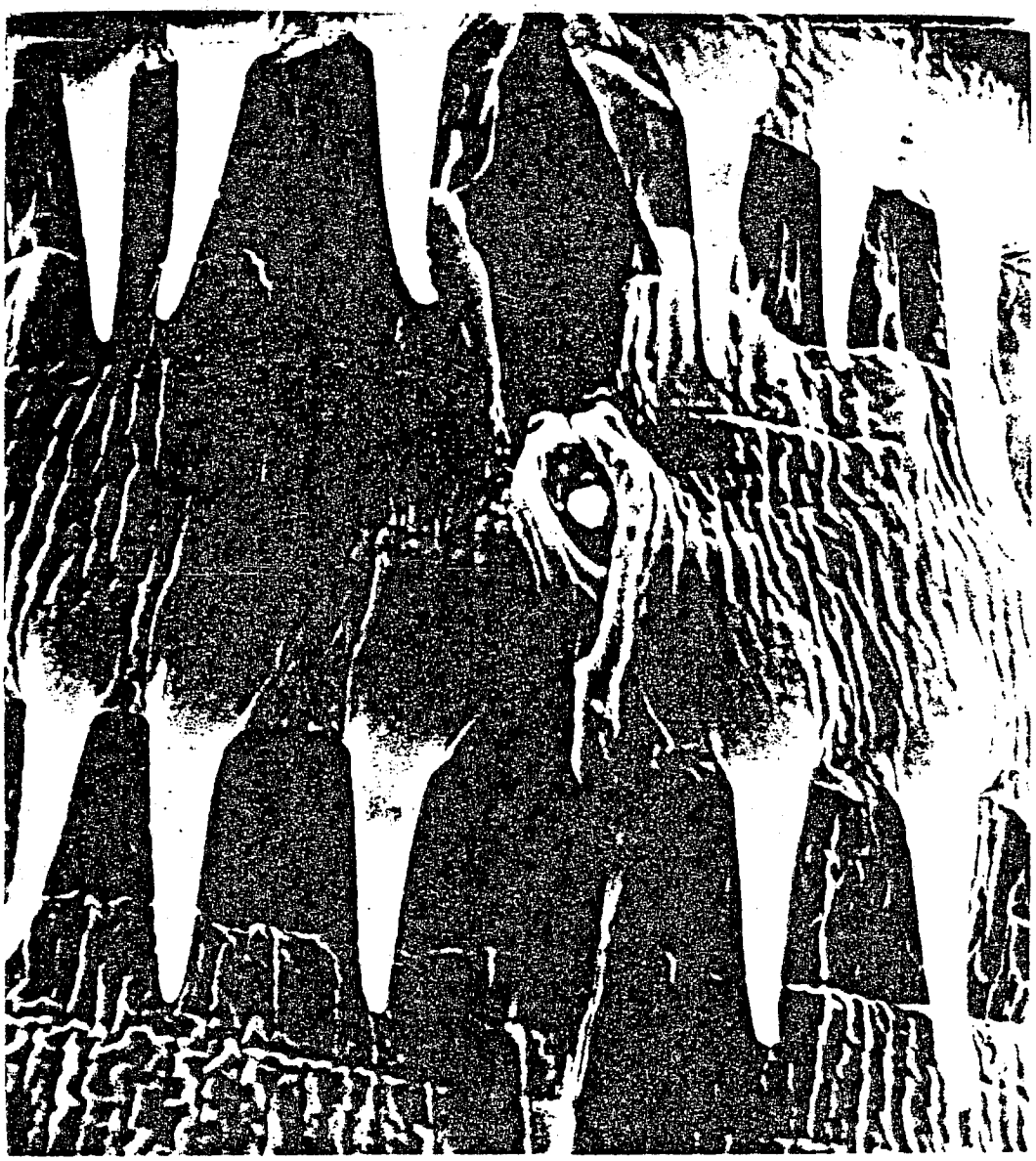

Figure 5. Deirid located between first and second rows of spines on L. carolini. (Bum ——————).

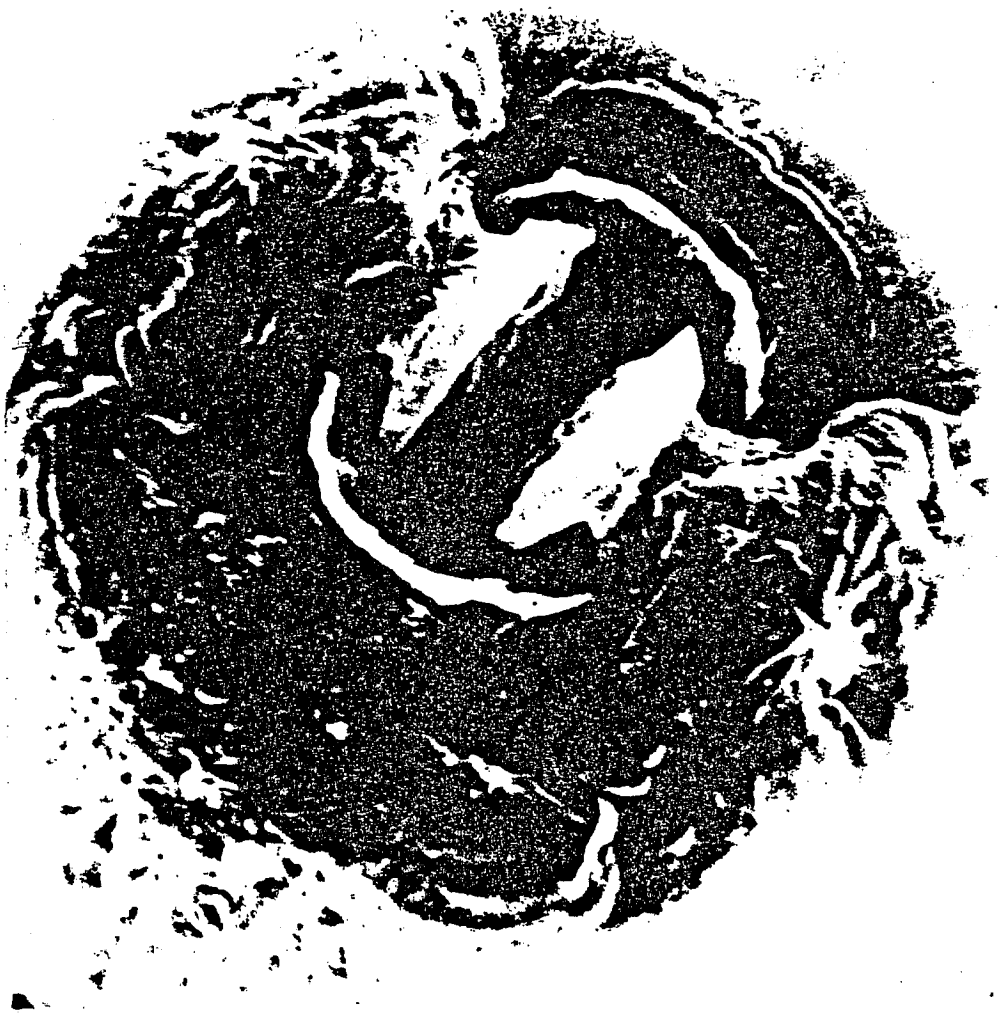

Figure 6. En face of S. carolini by SEM. Note 4 pairs of double cephalic papillae and double pored amphids. (5um ————————).

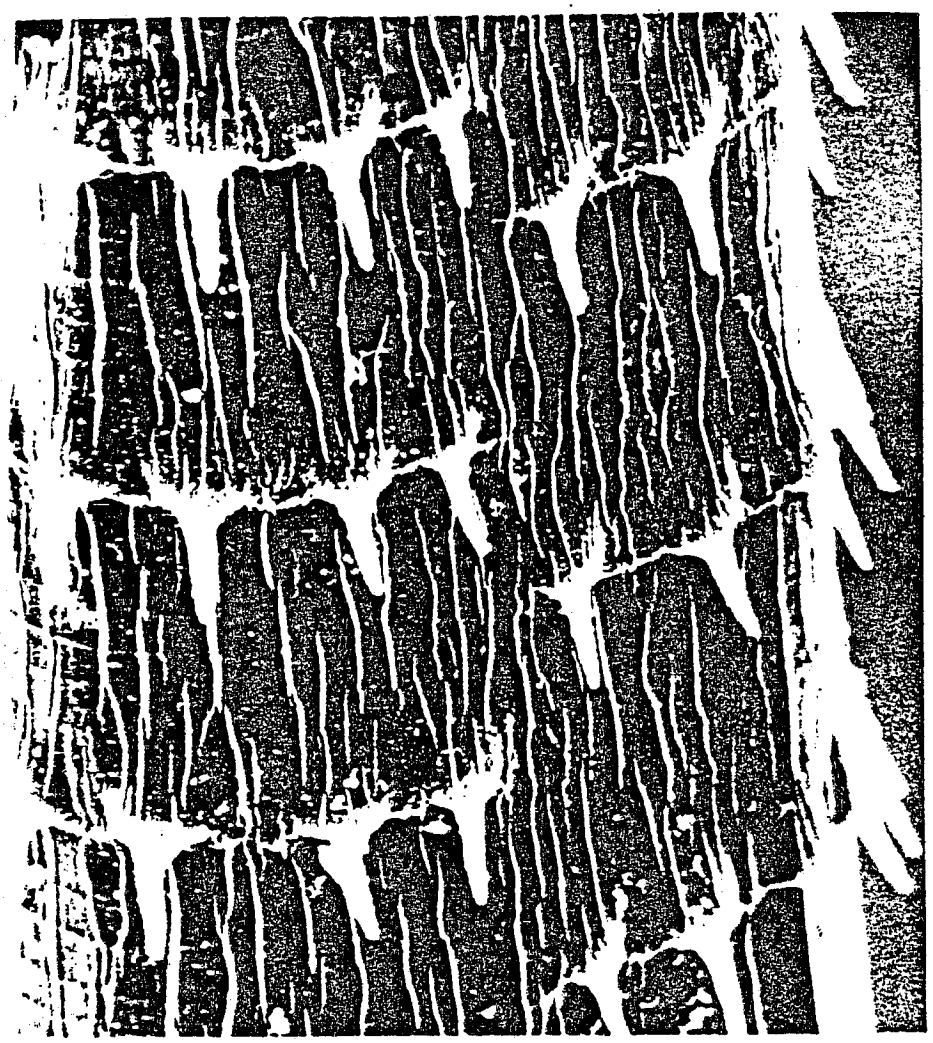

Figure 7. Spination along middle third of S. carolini SEM. Note break occurs along lateral line. (5um ————).

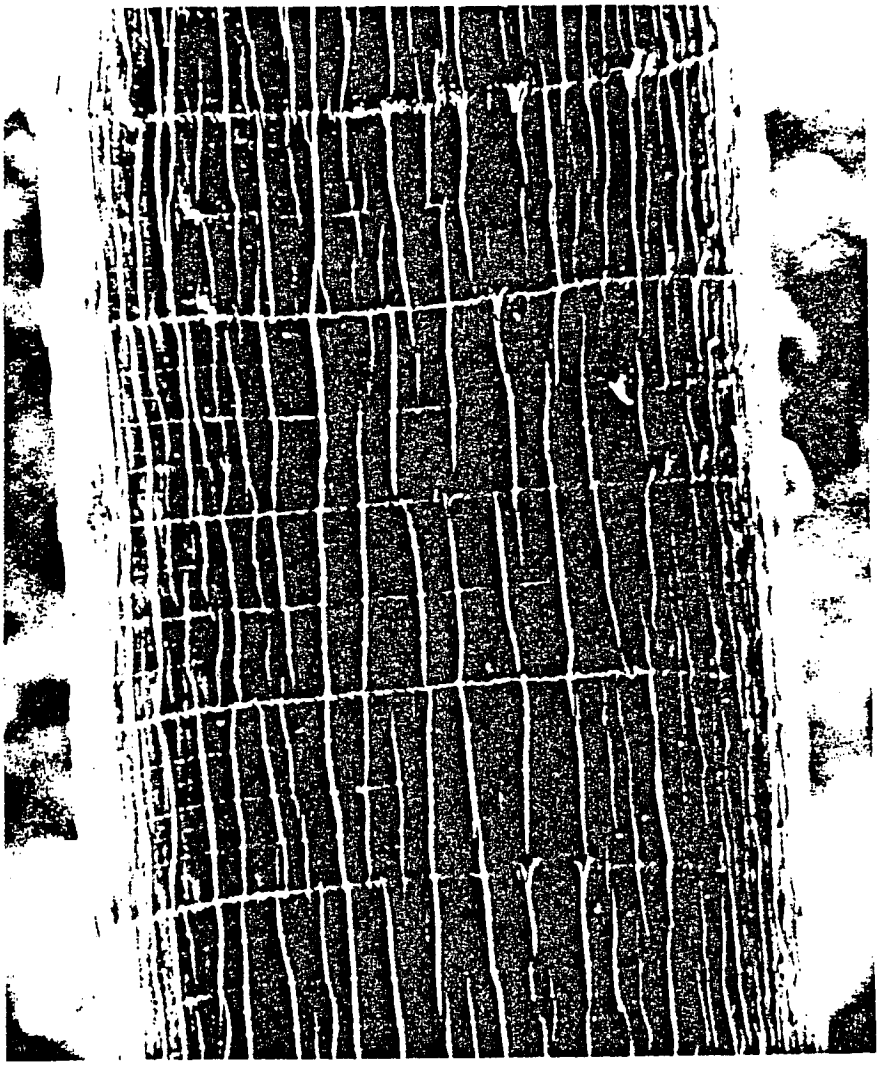

Figure 8. SEM of posterior one-third of *S. carolini*. Note infrequency and minuteness of spines. (5um ———).

with tail, proximally tubular, about half way along it becomes compressed, rounded, gutter like. Points of spicules lie slightly anterior to anus. Tip of tail is slightly rounded.

Female: Adult slender 4350 to 5700 (5133) long, 105 to 114 (110) wide. Nerve ring 140 to 156 (152) from anterior end. Muscular esophagus 168 to 235 (194) long, glandular esophagus 1437 to 1702 (1590) long. Vulva lies short distance posterior to middle of body. Ovejector well developed. Vagina approaching from rear. Gonoduct amphidelphic. Eggs, thickshelled. Tail of female bears mucron.

The fourth and final molt, never previously described for a species of Spinitectus, occurred 14 days post-infection of the fish definitive host. Further growth and development of the adults continued for 7 days. Sexually reproductive S. carolini occurred 21 days post-infection.

Figure 9. SEM of S. carolini egg. Egg heavily papillated. Eggs of S. gracilis similar. (5um ----------).

Plate I. Figs. 1-9. Life cycle of *Spinitectus carolini*. 1) zygote, 2) morula, 3) developing larva, 4) developing first stage larva, 5) first stage larva, 6) first stage larva molting to second stage larva, 7) early second stage larva, 8) late second stage larva, 9) third stage larva.

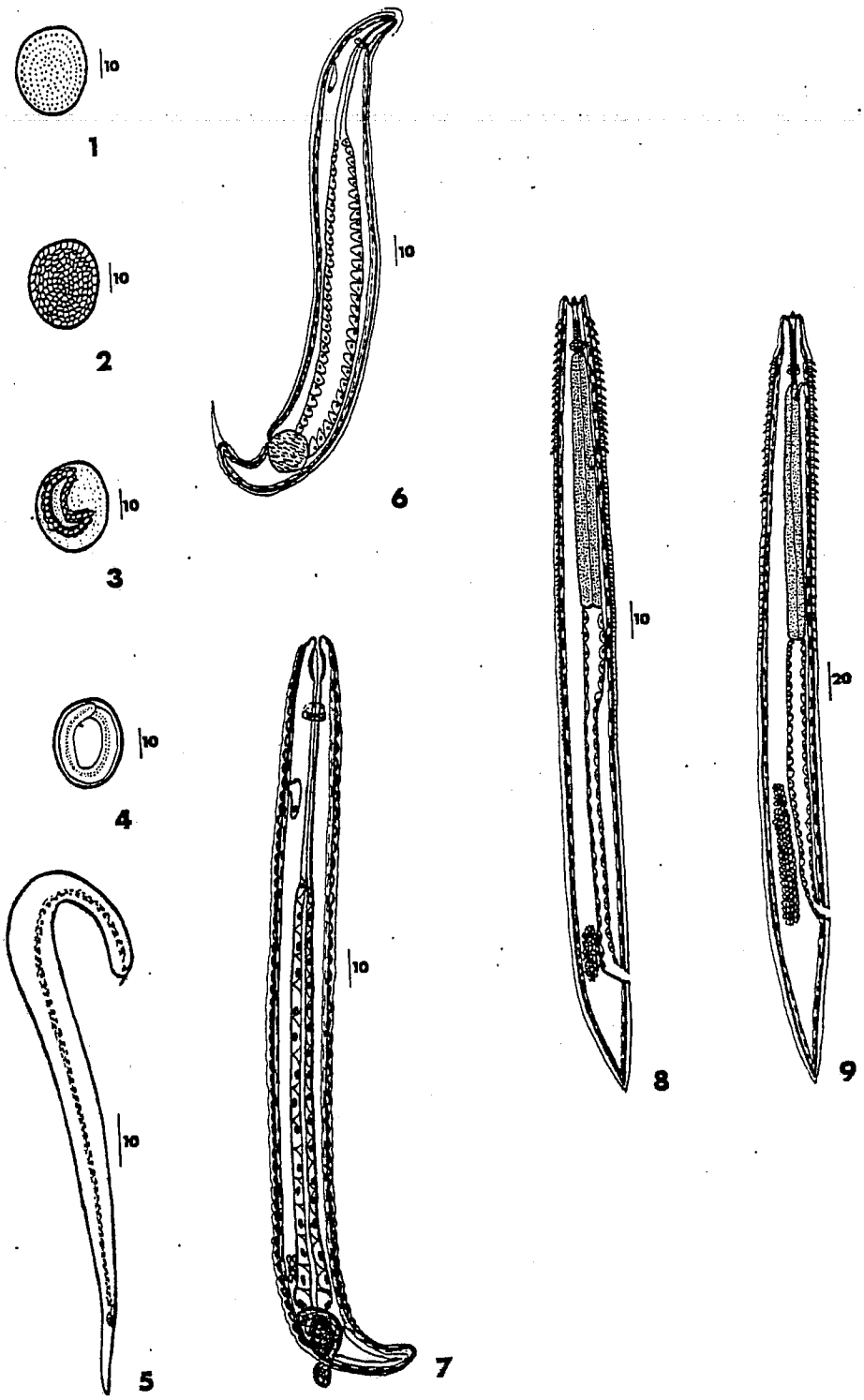
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Plate II. Figs. 10-18. Life cycle of Spinitectus carolini.

10) anterior end of adult, 11) ventral view, posterior end of adult male, 12) lateral view, posterior end of adult male, 13) lateral view, posterior end of female, 14) ventral view, mid-section of adult female (vagina, ovejector, uterus), 15) en face, second stage larva, 16) en face, third stage larva, 17) en face, fourth stage larva, 18) en face, adult.

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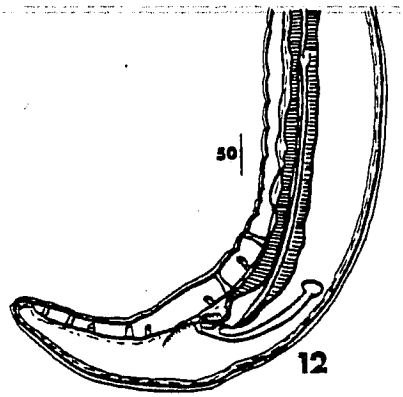

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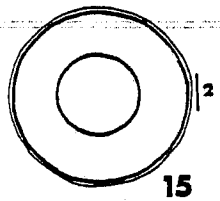

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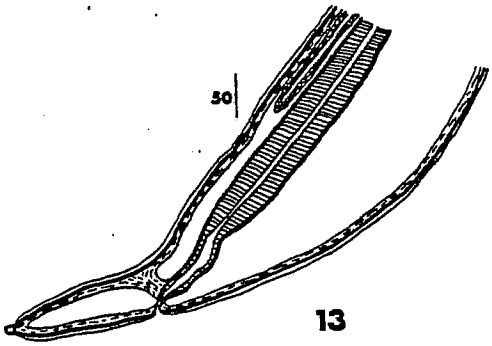

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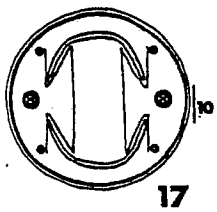

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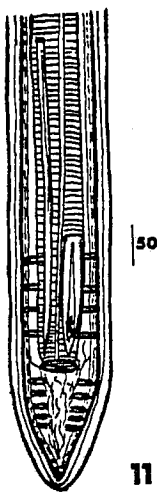

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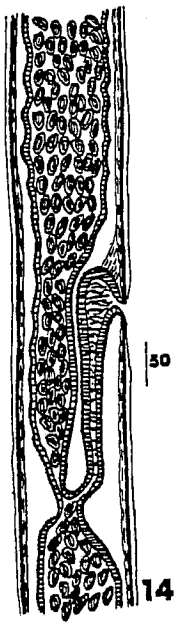

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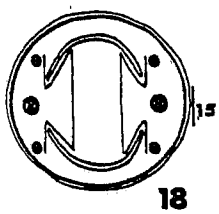

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Summary of the Experimental Life History (S. carolini)

1. Oviparous females released fully embryonated, larvated, eggs containing infective first stage larvae of Spinitectus carolini.

2. Larvated eggs were eaten by a variety of aquatic invertebrate hosts, however, infections occurred only in aquatic insect larvae. The larvae were released in the hosts gut and penetrated the gut wall within 6 hours post-infection, and entered the hemocoel.

3. The first molt began 18 hours post-infection within the hemocoel and terminated by hour 36 to yield the second stage larvae.

4. The second stage larvae remain in the hemocoel for 5 days where they underwent rapid development, which included the formation of the digestive tract, a buccal capsule, and spines.

The second stage larvae penetrated the abdominal muscles of the insect intermediate host and became encapsulated in host tissues.

6. The second molt to the thrid stage larvae occurred 8 days post-infection, within the abdominal muscle.

7. The third stage larvae was infective to the fish definitive host 8 days after the second molt, 14 days post-infection.

8. Infection of the fish definitive host was by ingestion of an infective intermediate host.

9. The third stage larvae remained in the intestinal lumen, or penetrated into the submucosa, or penetrated through the entire intestinal wall and encapsulated in the mesenteries surrounding the intestine.

10. The third molt occurred 2 days post-infection of the fish. The fourth stage larvae were characterized by continued growth and development of the genitalia and were distinguishable as males and females.

11. The fourth stage larvae began to molt 14 days post-infection. Further growth and development of the adult continued for 7 days, after which time fertilized female S. carolini were recovered.

THE LIFE HISTORY OF SPINITECTUS GRACILIS

Adult Spinitectus gracilis occurred in the pyloric caeca and in the intestinal tracts of fishes. Females were oviparous. Gravid females released larvated eggs which are subsequently voided from the digestive tract with the feces. The eggs then settled to the bottom.

The stages of development (Plate III, Figs. 1-4) occurred within the uterus of the female and yielded a first stage larva (Plate III, Fig. 5).

First stage larva (Plate III, Fig. 5).

First stage larvae slender 335 to 352 (Mean 340) long, 25 to 34 (31) wide. Anterior end rounded, has pointed egg tooth. Stoma indistinct. Nerve ring 43 to 56 (46) from anterior end. Excretory pore 75 to 78 (76) from anterior end. Esophagus thin walled 120 to 165 (142) long. Intestine indistinct, four rectal cells, anal plug present. Genital primordium composed of 6 cells, 263 to 303 (272) from anterior end. No mucrones present.

Determination of the intermediate host and larval development.

Intermediate hosts were infected as prescribed in the materials and methods. Spinitectus gracilis infections occurred in mayfly naiads (Baetis, Caenis, Ephemerella, Heptagenea, Hexagenia, and Stenonema), dragonfly nymphs (Gomphus and Pachydiplax), stonefly larvae (Isch-

Table III. EXPERIMENTAL DETERMINATION OF THE INTERMEDIATE HOST OF *SPINITECTUS GRACILIS* AND NATURAL INFECTIONS.

Potential Host	Natural Infection	Experimental Infection
Hexagenia	+	+
Caenis	+	+
Stenonema	-	+
Heptagenia	+	+
Baetis	-	+
Ephemerella	-	+
Gomphus	-	+
Pachydiplax	-	+
Ischnura	-	+
Neoperla	+	-
Acroneuria	-	+
Belostoma	-	-
Notonecta	-	-
Limnephilus	-	-
Keratella	-	-
Chironomus	-	-
Asellus	-	-
Gammurus	-	-
Arrenurus	-	-
Ostracods	-	-
Diaptomus	-	-
Cyclops	-	-
Bosmina	-	-
Daphnia	-	-
Chaetogaster	-	-
Branchiura	-	-
Podura	-	+

nura and Neoperla), and collembolans (Podura). Information pertaining to natural and experimental infections of intermediate hosts is presented in Table III.

First stage larvae hatched from the eggs within the midgut of the insect intermediate host. The larvae penetrated through the midgut wall within the first 6 hours and entered the insects hemocoel. The first molt began 18 hours post-infection within the hemocoel and terminated by hour 36 to yield a second stage larva. A considerable degree of development occurred just prior to and just after the first molt. The stoma became oval shaped. The buccal capsule formed. The esophagus became thicker walled and differentiated into two parts, a muscular and a glandular portion. The intestinal lumen appeared. The genital primordium increased in cell number. Body spines appeared.

Second stage larva (Plate III, Figs. 6 & 7)

Second stage larvae slender 453 to 507 (471) long, 31 to 36 (33) wide. Nerve ring 92 to 97 (95) from anterior end. Excretory pore 97 to 108 (101) from anterior end. Thick walled esophagus composed of two parts: glandular 105 to 109 (108) long and muscular 71 to 88 (79) long. Intestinal lumen present, has distinct cell lining. Genital primordium 397 to 414 (407) from anterior end. Four rectal cells visible surrounding anus. No mucrones present. Spines on anterior one-fourth of body.

The second stage larvae grew and developed within the hemocoel for 6 days, after which they penetrated the abdominal muscles of the insect intermediate hosts. The larvae were encapsulated by host tissues. The second molt to the third stage occurred 8 days post-infection within the encapsulation in insects abdominal muscle tissue.

Third stage larva (Plate III, Fig. 8)

Third stage larvae slender 1721 to 1887 (1779) long, 40 to 43 (41) wide. Anterior end rounded. Oral aperature terminal, two lateral pseudolips, two medial amphids on pseudolips. Four small papillae, two subventral, two subdorsal. Nerve ring 108 to 116 (112) from anterior end. Excretory pore 151 to 157 (155) from anterior end. Esophagus 915 to 954 (945) long, muscular region 189 to 194 (192) long, glandular region 726 to 760 (753) long. Genital primordium 1149 to 1305 (1233) from anterior end, increased substantially in cell number.

The third stage larvae were as the second stage larvae, encapsulated in the abdominal muscle tissues of the insect intermediate hosts. The third stage larvae were characterized by an increased size and development of the genital primordium. The capsules were impregnated with melanin (Jilek and Crites, 1980a) as they persisted in the abdominal muscles and were seen easily through the body wall. The larvae remained in a quiescent state. The number and shape of the spines increased and were di-

agnostic for the species. Deirids were seen between the first and second rows of spines, along the lateral lines. Mucrones were present, and served as a means of differentiating females from males, as mucrones were only present on female S. gracilis. The stoma assumed the characteristic appearance of the adult.

Determination of the definitive host

The definitive host (green sunfish, Lepomis cyanellus) were infected with third stage larvae of S. gracilis as prescribed in the materials and methods. Third stage larvae were infective to the fish definitive host 15 days post-infection of the intermediate host.

Fourth stage larva

Oral vestibule short and bent at right angle. Two lateral pseudolips, each with single amphid containing a double pore. Four papillae, two subventral, two subdorsal. Deirids present between first and second rows of spines. No spines over lateral lines, point at which rows of spines break. Spines occur over entire body surface, fewer and more dispersed from anterior to posterior.

Male: Fourth stage larvae slender 2575 to 2980 (2767) long, 75 to 98 (89) wide. Nerve ring 126 to 139 (135) from anterior end. Excretory pore 165 to 198 (183) from anterior end. Esophagus 1180 to 1356 (1297) long, muscular region 159 to 187 (175) long, glandular region 1021 to 1169 (1072) long. Narrow alae present posteriorly. Four pairs of pedunculate preanal and five pairs of pedunculate postanal papillae. Two spicules, right short,

left long. No gubernaculum. Testis single reflexed, extends anteriorly to level of separation of muscular and glandular esophagus. Parallel cuticular striations absent around anal region.

Female: Fourth stage larvae slender 2896 to 3375 (3156) long, 85 to 94 (93) wide. Nerve ring 126 to 139 (135) from anterior end. Excretory pore 165 to 198 (183) from anterior end. Esophagus 1480 to 1829 (1701) long, muscular region 198 to 246 (229) long, glandular region 1258 to 1575 (1471) long. Vulva in middle of body. Ovejector well developed. Vagina approaches ovejector from anterior. Uterus amphidelphic. Mucron present.

After being freed from the capsule, within the intestine of the fish, the infective third stage larva subsisted in the intestinal lumen, penetrated the intestinal wall and subsisted in the submucosa, or penetrated through the entire intestinal wall and became encapsulated by host tissues in the mesenteries surrounding the intestine. The third molt to the fourth stage larva occurred 2 days post-infection of the fish definitive host in each of the three distinct microhabitats (intestinal lumen, submucosa, or mesenteric encapsulation). The fourth stage larvae underwent rapid growth and development including differentiation of the gonadal tissues. Male structures such as: spicules, single reflexed testis, and caudal papillae were visible. Female development included:

formation of the vulva, uterus, ovejector and vagina.

Adult (Plate IV, Figs. 9, 10, 11, 12, 13, & 17)

Short oral vestibule, exhibiting sharp bend, ending in posterior third of head cone. Vestibule is joined by muscular esophagus. Diameter of muscular esophagus is one-half diameter of glandular esophagus. Nerve ring is located between the second and third rows of spines. Excretory pore situated between fourth and fifth rows of spines. Spines short and sharp, with 25 to 35 per row. Discontinuity in rows of spines over the lateral lines, more marked further posterior. Spines occur on projections of cuticula, decrease in number, size from anterior to posterior. Head bears 4 double papillae, two lateral amphids situated laterally on pseudolips. Each amphid bears two pore like openings. Deirids lateral, between first and second rows of spines.

Male: Adults slender 3852 to 4390 (4150) long, 102 to 130 (117) wide. Nerve ring 142 to 155 (150) from anterior end. Excretory pore 203 to 246 (221) from anterior end. Muscular esophagus 203 to 303 (257) long, glandular esophagus 1607 to 1895 (1805) long. Narrow alae present. Four pedunculate preanal and five pedunculate postanal papillae on eith r side of tail. Right spicule short and thick, with 90 degree bend and thickened proximal portion, distal end is pointed and lacks characteristic barb of S. carolini. Left spicule long, slender, no change in form.

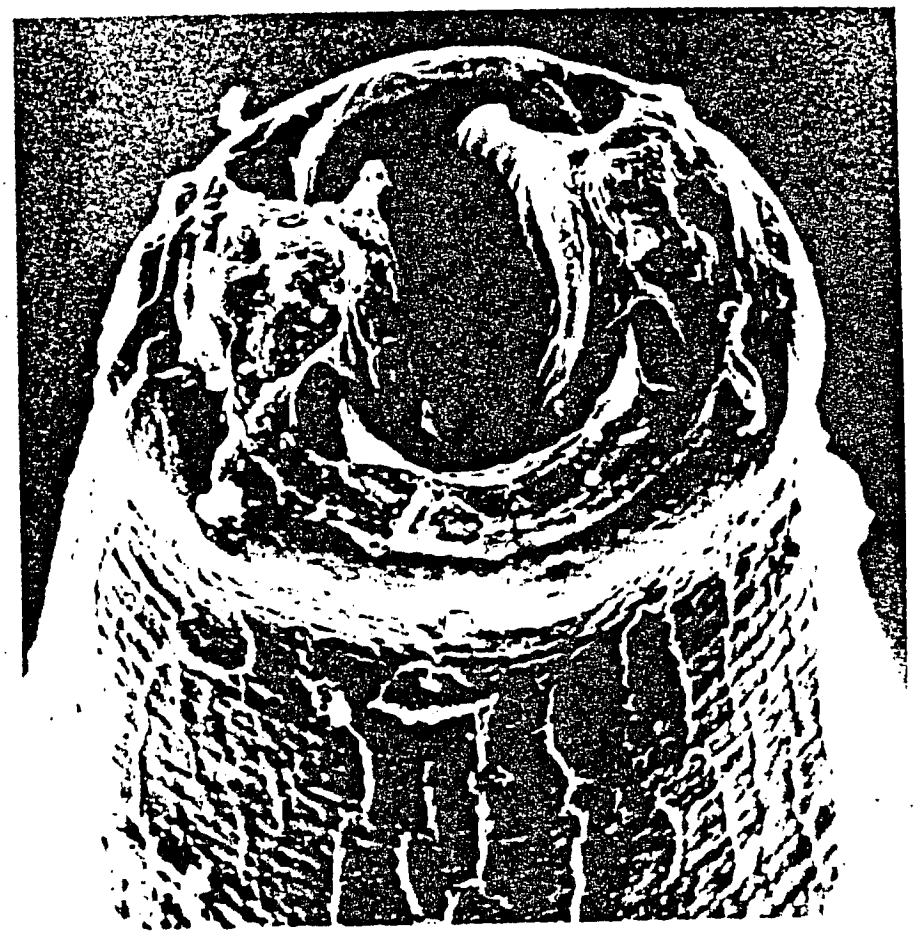

Figure 10. SEM of en face of S. gracilis. En face views of adult S. carolini and S. gracilis identical. (5um ————————).

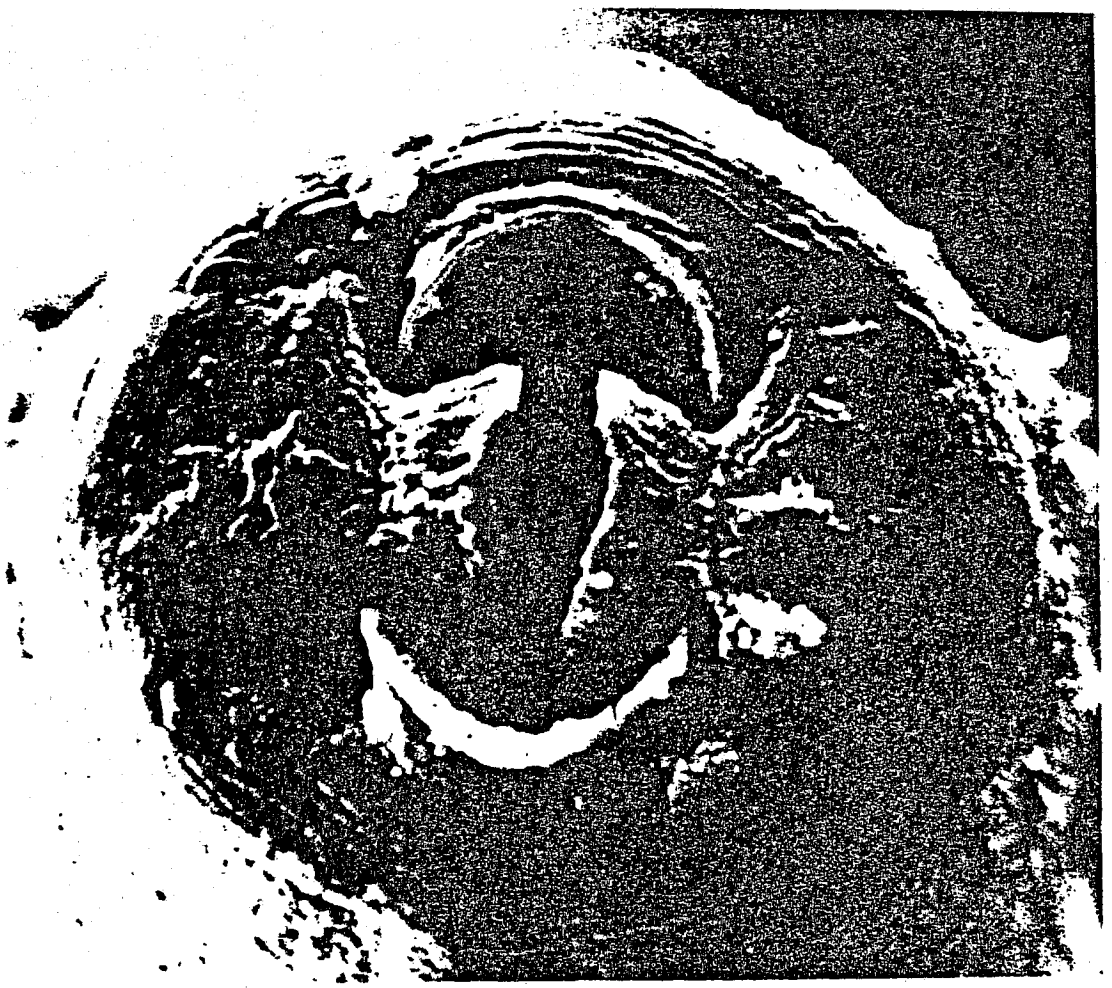

Figure 11. En face of adult S. gracilis. Note 4 double papillae, two subventral, two subdorsal. Also amphids on pseudolips. SEM. (5um ——————).

Figure 12. SEM of deirid between first and second rows of spines on S. gracilis, deirid closed. (5um ----------).

Figure 13. SEM enlargement of S. gracilis deirid in an open state. Note the single central projection. (.5um ————).

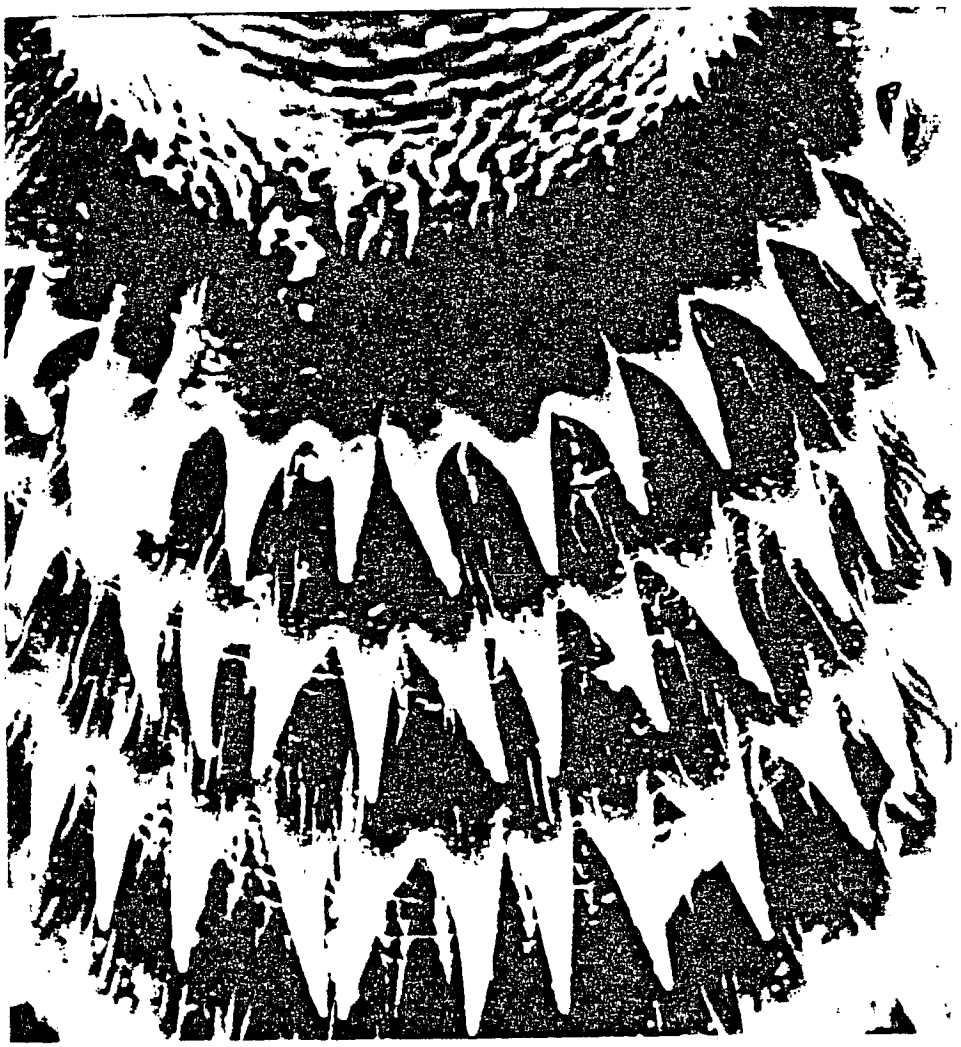

Figure 14. SEM of anterior three rows or spines of S. gracilis. Note spines in groups of 2 and 3. (5um ----------).

Figure 15. SEM of spines from rows 4 and 5 of S. gracilis. Note spines are single rather then in three's and two's. (5um———————)

Points of spicules lie anterior to anal opening. No gubernaculum. Tip of tail rounded.

Female: Adult slender 5648 to 7390 (6250) long, 147 to 163 (155) wide. Nerve ring 153 to 167 (160) from anterior end. Excretory pore 202 to 235 (228) from anterior end. Muscular esophagus 184 to 243 (229) long, glandular esophagus 1784 to 2075 (1903) long. Vulva lies short distance posterior to middle of body. Ovejector is well developed. Vagina approaches from anterior. Gonoduct amphidelphic. Eggs thickshelled. Tail of female bears a mucron.

The fourth and final molt, never previously described for a species of Spinitectus was found. It occurred 15 days post-infection of the fish definitive host. Further growth and development of the adults continued for 8 days. Sexually reproductive S. gracilis occurred 24 days post-infection.

Plate III. Figs. 1-8. Life cycle of Spinitectus gracilis 1) zygote, 2) morula, 3) developing larva, 4) developing first stage larva, 5) first stage larva, 6) first stage larva molting to second stage larva, 7) second stage larva, 8) third stage larva.

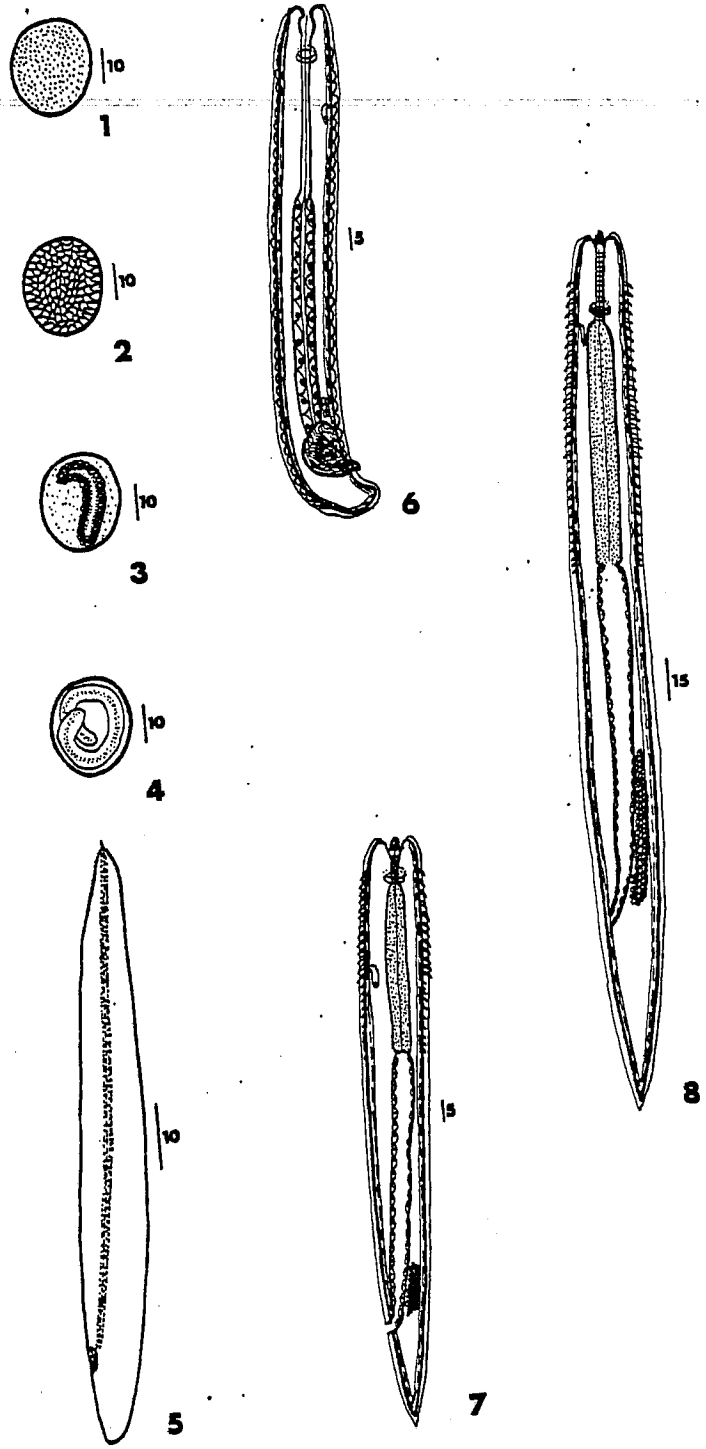
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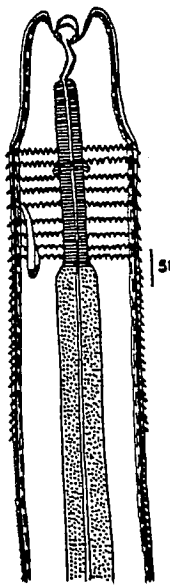
Plate IV. Figs. 9-17. Life cycle of Spinitectus gracilis 9) anterior end of adult, 10) ventral view, posterior end of adult male, 11) lateral view, posterior end of adult male, 12) lateral view, posterior end of adult female, 13) ventral view, midsection of adult female (vagina, ovejector, uterus), 14) en face, second stage larva, 15) en face, third stage larva, 16) en face, fourth stage larva, 17) en face, adult.



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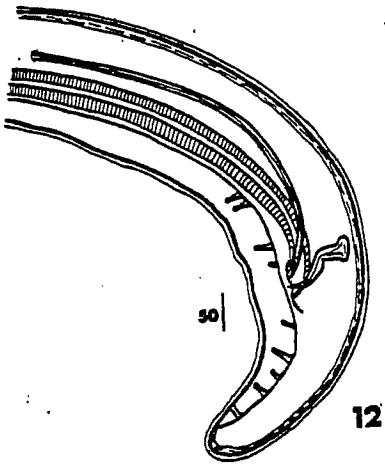

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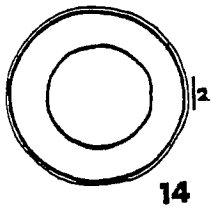

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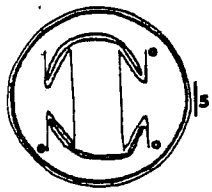

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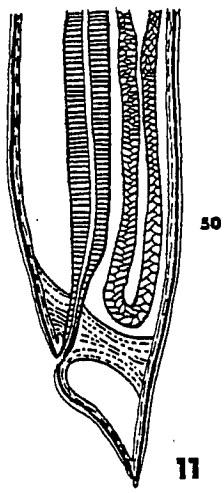

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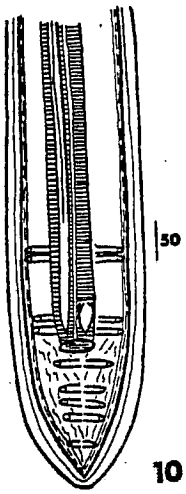

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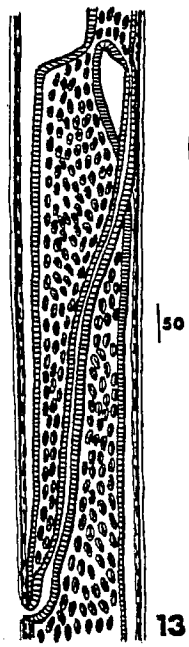

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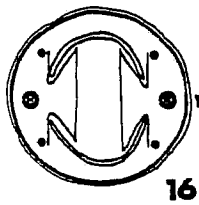

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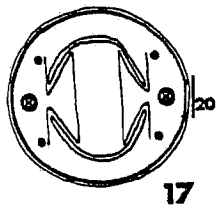

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Summary of the Experimental Life History (S. gracilis)

1. Oviparous females released larvated eggs con-infective first stage larvae of Spinitectus gracilis.

Larvated eggs were eaten by a variety of aquatic invertebrates, however, infections occurred only in aquatic insect larvae. The larvae were released in the hosts gut and penetrated the gut wall within 6 hours post-infection, and subsequently entered the hemocoel.

2. The first molt began 18 hours post-infection within the hemocoel and terminated by hour 36 to yield the second stage larvae.

3. The second stage larvae remained in the hemocoel for 6 days where they underwent rapid development, which included the formation of the digestive tract, a buccal capsule, and spines.

4. The second stage larvae penetrated the abdominal muscles of the insect intermediate host and became encapsulated in host tissues.

5. The second molt to the third stage larvae occurred 8 days post-infection, within the abdominal muscle.

6. The third stage larvae was infective to the fish definitive host 8 days after the second molt, 15 days post-infection.

7. Infection of the fish definitive host was by ingestion of an infected intermediate host.

8. The third stage larvae remained in the intestinal lumen, or penetrated into the submucosa, or penetrated through the entire intestinal wall and encapsulated in the mesenteries surrounding the intestine.

9. The third molt occurred 2 days post-infection of the fish. The fourth stage larvae were characterized by continued growth and development of the genitalia and were distinguishable as males and females.

10. The fourth stage larvae molted to adults 15 days post-infection. Further growth and development of the adult continued for 8 days, after which time fertilized female S. gracilis were recovered.

PREVALENCE AND HOST SPECIFICITY

Intermediate host specificity was confined primarily to the insect orders: Diptera, Ephemeroptera, Odonata, Plecoptera, for both Spinitectus carolini and Spinitectus gracilis (Tables II & III). Natural infections of S. carolini were found in 6 of 107 chironomid larvae (Chironomus) examined. Experimental infections of chironomid larvae with larvated eggs of S. carolini and S. gracilis were negative. Success was evidenced in infecting 3 of 5 collembolans (Podura) experimentally with S. gracilis. Attempts to infect numerous other invertebrate animals such as: crustaceans, annelids, rotifers, ...etc. were unsuccessful. Fully developed, infective, third stage larvae of S. carolini and S. gracilis were recovered from all naturally and experimentally infected hosts.

Little host specificity was observed within the definitive fish hosts (Tables IV & V). Eleven species of important game fish were examined from the western basin of Lake Erie for the prevalence of S. carolini and S. gracilis (Tables IV & V). When the present study was compared with that of Bangham and Hunter (1939) and Bangham (1972), one encountered increases in S. carolini in: Ictalurus nebulosus and Ictalurus punctatus; decreases in S. carolini prevalence in: Ambloplites ru-

Table IV. Prevalence of S. carolini and S. gracilis in 11 species of Lake Erie game fish, along with Bangham & Hunter's study (1927-1929) and Bangham's study (1957).

Species of Fish		Prevalence 1927--1929	Prevalence 1957	Prevalence 1978--1979[a]
Ambloplitis rupestris				
	S. carolini	4/12 33.3%	75/75 100.0%	421/523 80.5%
	S. gracilis	0/12 0.0%	0/75 0.0%	160/523 30.6%
Ictalurus nebulosus				
	S. carolini	(None collected)	0/47 0.0%	3/42 7.1%
	S. gracilis		5/47 10.6%	4/42 9.5%
Ictalurus punctatus				
	S. carolini	0/29 0.0%	0/39 0.0%	4/49 8.2%
	S. gracilis	4/29 13.8%	14/39 35.9%	8/49 16.3%
Lepomis gibbosus				
	S. carolini	1/23 4.3%	38/58 65.5%	76/238 31.9%
	S. gracilis	0/23 0.0%	0/58 0.0%	79/238 33.2%
Lepomis macrochirus				
	S. carolini	0/10 0.0%	69/74 93.2%	93/226 41.2%
	S. gracilis	0/10 0.0%	0/74 0.0%	57/226 25.2%
Micropterus dolomieu				
	S. carolini	5/80 6.25%	28/51 54.9%	31/142 21.8%
	S. gracilis	0/80 0.0%	0/51 0.0%	55/142 38.7%
Micropterus salmoides				
	S. carolini	0/129 0.0%	7/40 17.5%	3/38 7.9%
	S. gracilis	0/129 0.0%	0/40 0.0%	4/38 10.5%
Morone chrysops				
	S. carolini	0/29 0.0%	1/53 1.9%	0/50 0.0%
	S. gracilis	0/29 0.0%	0/53 0.0%	3/50 6.0%
Perca flavescens				
	Spinitectus sp.	0/60 0.0%	5/93 5.4%	0/69 0.0%
	S. carolini	0/60 0.0%	0/93 0.0%	6/69 8.8%
	S. gracilis	0/60 0.0%	0/93 0.0%	0/69 0.0%
Pomoxis annularis				
	S. carolini	0/17 0.0%	0/53 0.0%	0/56 0.0%
	S. gracilis	0/17 0.0%	10/53 18.9%	0/56 0.0%
Pomoxis nigromaculatus				
	S. carolini	0/09 0.0%	0/29 0.0%	0/44 0.0%
	S. gracilis	0/09 0.0%	11/29 37.9%	0/44 0.0%

a-present study

Prevalence of Spinitectus (1978-9)

Species	Ex.	%un	%Sc	%Sg	%S
Ambloplitis rupestris	533	12	79	20	21
Ictalurus nebulosus	42	88	6	9	3
I. punctatus	49	77	8	17	2
Lepomis gibosus	228	44	32	33	10
L. macrochirus	226	50	41	25	16
Micropterus dolomieu	142	45	22	39	16
M. salmoides	38	87	8	9	4
Morone chyrsops	50	94	0	6	0
Perca flavescens	69	91	9	0	0
Pomoxis annularis	36	100	0	0	0
P. macrolatus	44	100	0	0	0

Table V. Prevalence of Spinitectus in 11 species of game fishes collected from the western basin of Lake Erie (1978-1979). Ex-examined; un-uninfected fish; Sc-Spinitectus carolini; Sg-Spinitectus gracilis; S-dual infections (S. carolini and S. gracilis).

pestris, Lepomis gibbosus, Lepomis macrochirus, Micropterus dolomieui, Micropterus salmoides, Morone chrysops, and Perca flavescens. There was an increase in the prevalence of S. gracilis in: Ambloplites rupestris, Lepomis gibbosus, Lepomis macrochirus, Micropterus dolomieui, Micropterus salmoides, and Morone chrysops; while there were decreases in the prevalence of S. gracilis in Ictalurus punctatus, Pomoxis annularis, and Pomoxis nigromaculatus.

Dual infections of fish with S. carolini and S. gracilis were observed for the first time. These dual infections occurred in: Ambloplites rupestris, Ictalurus nebulosus, Ictalurus punctatus, Lepomis gibbosus, Lepomis macrochirus, Micropterus dolomieui, and Micropterus salmoides.

The only fish found to be completely uninfected with either S. carolini or S. gracilis were the black crappie, Pomoxis nigromaculatus, and white crappie, Pomoxis annularis.

New host records for S. carolini were found in I. punctatus, while for S. gracilis new host records were found in A. rupestris, L. gibbosus, L. macrochirus, M. dolomieui, M. salmoides, P. flavescens, and M. chrysops (Table IV).

The host specificity of S. carolini and S. gracilis in Lake Erie appeared very broad, and may be depen-

dent on encounters with the appropriate larval stages. Encounters may occur either through the consumption of an intermediate host infected with larval Spinitectus or by predation of one fish on another. Successful transfers of third stage, fourth stage, and adult S. carolini and S. gracilis from one fish to another led to the conclusion of the predator/prey infection route.

PATHOLOGY

The pathology associated with Spinitectus carolini and Spinitectus gracilis infections occurs at two separate levels. One level represented the insect intermediate host pathology, while the second level represented the definitive host pathology. The extent of pathology was directly proportional to the number of parasites in the host organism.

Numerous aquatic insect larvae were utilized (Table I), however, for descriptive purposes, only that pathology produced by Spinitectus in mayfly larvae, Hexagenia, will be discussed. Similar results were observed in each group of insects infected with Spinitectus. Larvated Spinitectus eggs were ingested by mayfly naiads. The first stage larvae were released from the eggs within the midgut. Here the larvae penetrated into (Fig. 3) and ultimately through the midgut wall. Once through the larvae grew and molted to the second stage larvae within the naiads hemocoel. The extent of damage created by the larval nematodes within the hemocoel could not be determined, but it is postulated that their extreme rate of growth may deplete some of the stored energy reserves of the naiad.

The second stage larvae penetrated into the abdominal muscles of the naiads six days post-infection. At this time the host naiad mounted a defensive reaction that culminated in the encapsulation of the second stage larvae

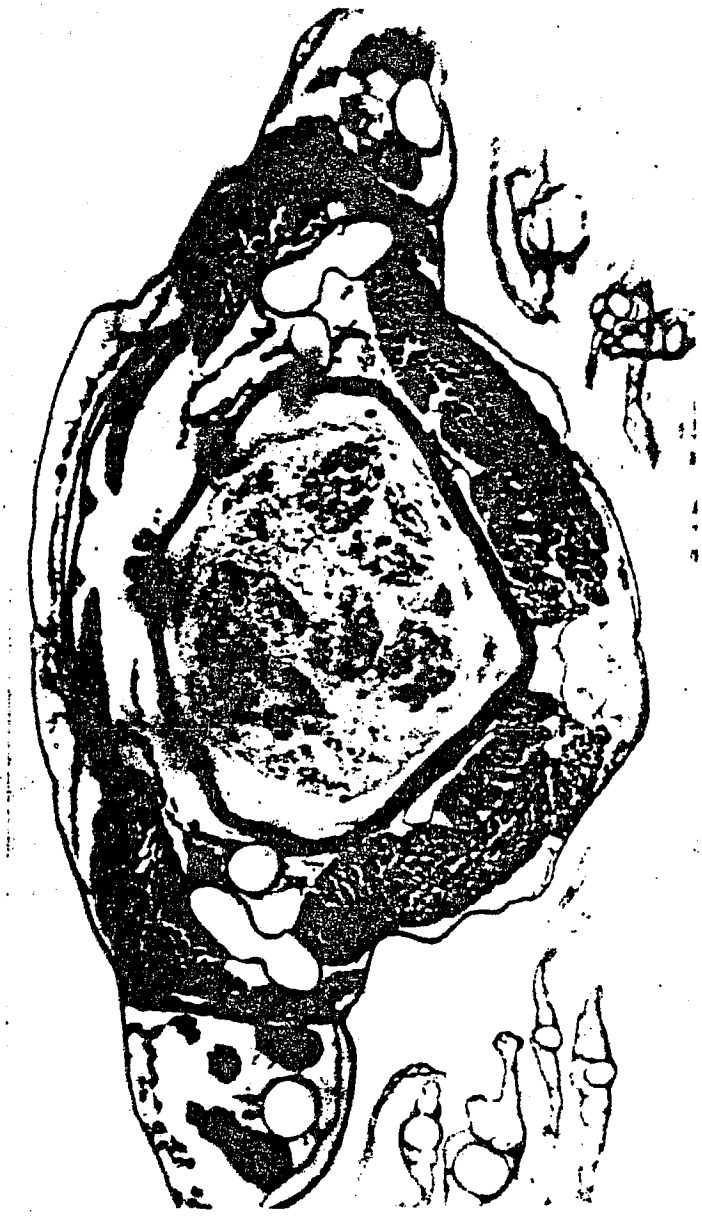

Figure 16. Normal histology of mayfly naiad, Hexagenia limbata. 40X.

Figure 17. Photomicrograph of mayfly naiad, Hexagenia limbata, infected with S. carolini larvae. Note encapsulated S. carolini in abdominal muscles. 40X.

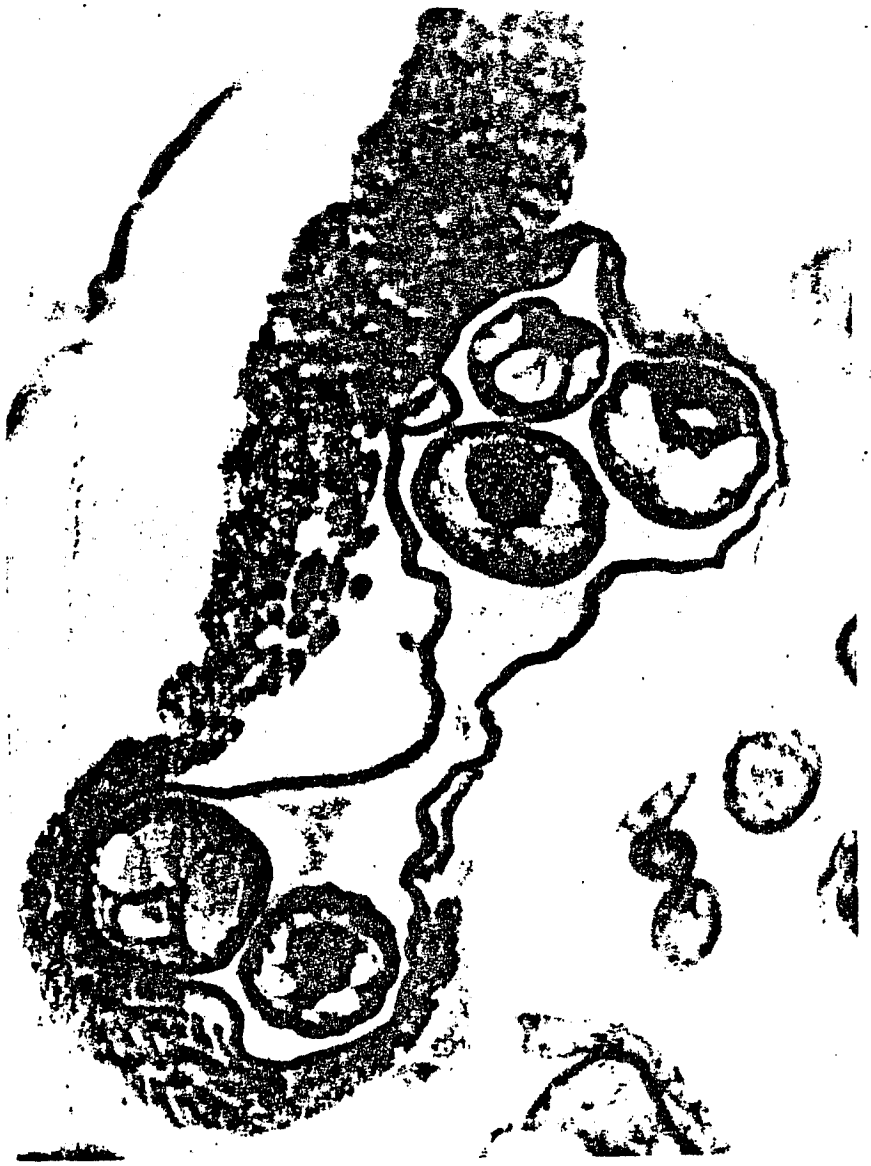

Figure 18. Photomicrograph of S. carolini larva encapsulated in abdominal muscle tissue of Hexagenia limbata. 100X.

(Jilek and Crites, 1980a). The larvae continued to grow within the capsule and molted eight days post-infection to third stage larvae. As the larvae grew and developed, so also did the capsule. Mechanical pressure produced by the increased size of the capsule aided in destroying abdominal muscle tissue (Figs. 17 & 18).

The capsule eventually was impregnated with a brownish yellow pigment. Histochemical characterization of the pigment utilized three different stains (ferrous iron, toluidine blue, and Giemsa) indicated the presence of melanin in the capsule wall, which attributed to its brownish yellow appearance (Jilek and Crites, 1980).

Experimental infections showed the naiads capable of harboring in excess of 100 larval Spinitectus, after an extended exposure period of four weeks to larvated eggs. Larval Spinitectus remained viable for the duration of the experimental period (three months). No evidence was observed that would indicate death to Spinitectus larvae or the naiad, in either light or heavy infections. However, the maximum number of third stage larvae recovered from experimentally infected naiads which metamorphosed to adults or naturally infected adult Hexagenia was 8, therefore suggesting the inability of naiads to metamorphose or survive metamor-

phosis when infected with larger numbers of Spinitectus larvae (Jilek and Crites, 1980a). This was not inexplicable when one considered the extensive damage produced by the larvae to the abdominal muscle tissue of the naiads. The abdominal muscles were instrumental in both the larval and adult locomotion (Figs. 16 & 17).

After a six day period of growth and development within the mayfly naiad the third stage larvae were infective to the fish definitive host. In many infections of the intestine the lesions, particularly of the mucosa, were striking. Spinitectus were released from their encapsulations in the intestines of the fish. Here it appeared the third stage larvae either remained in the intestinal lumen in close apposition to the mucosa, or penetrated through the mucosa and entered the submucosa, or lastly penetrated through the entire intestine (mucosa, submucosa, muscularis externa, and serosa) and encapsulated in the mesenteries surrounding the intestine (Figs. 19, 20, 21, & 22). The intestinal lumen microhabitat was the most common (approximately 80%), while the submucosal microhabitat and mesenteric encapsulation microhabitat were the least frequent (approximately 10% each) (Jilek and Crites, 1980b).

Development of the third stage larvae to adult Spinitectus occurred in each of the three microhabitats. Development included two more molts, the third and fourth,

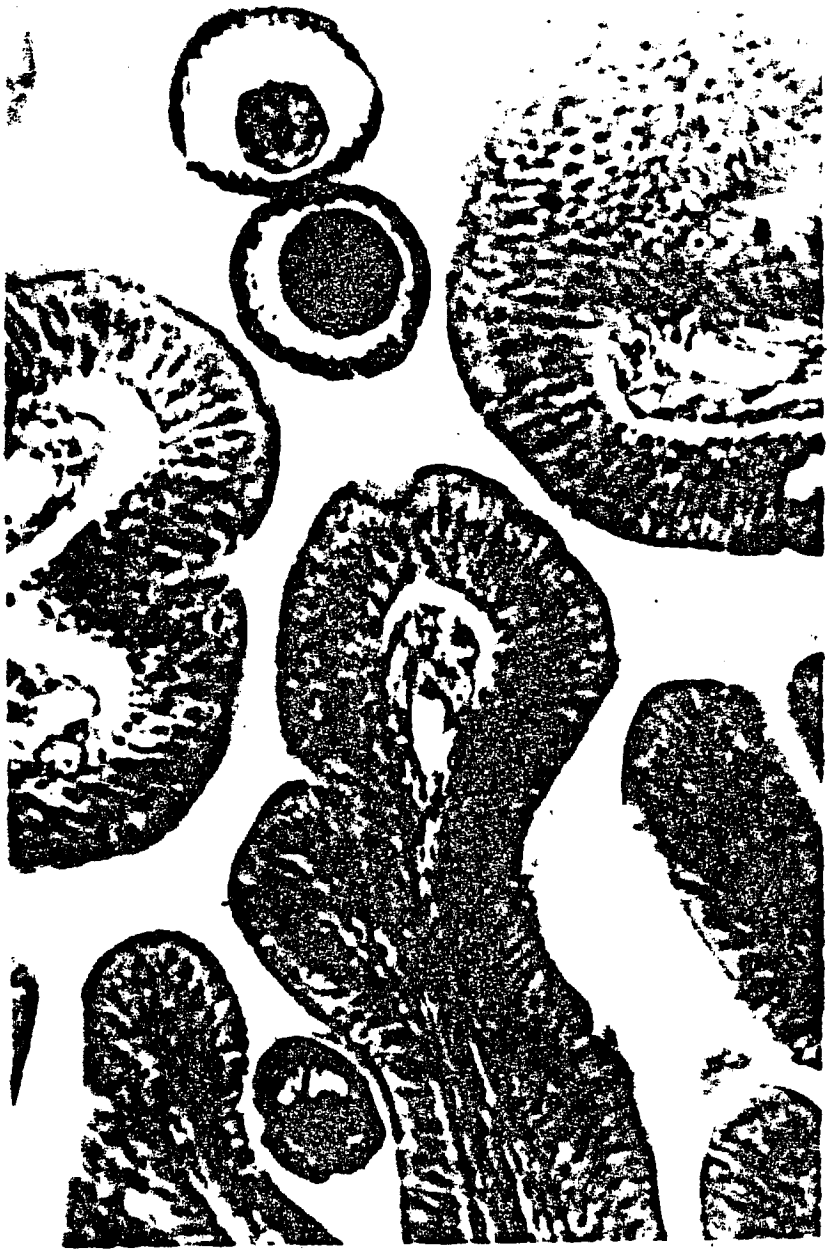

Figure 19. Light photomicrograph of S. gracilis in the intestinal lumen of a bluegill. 100X.

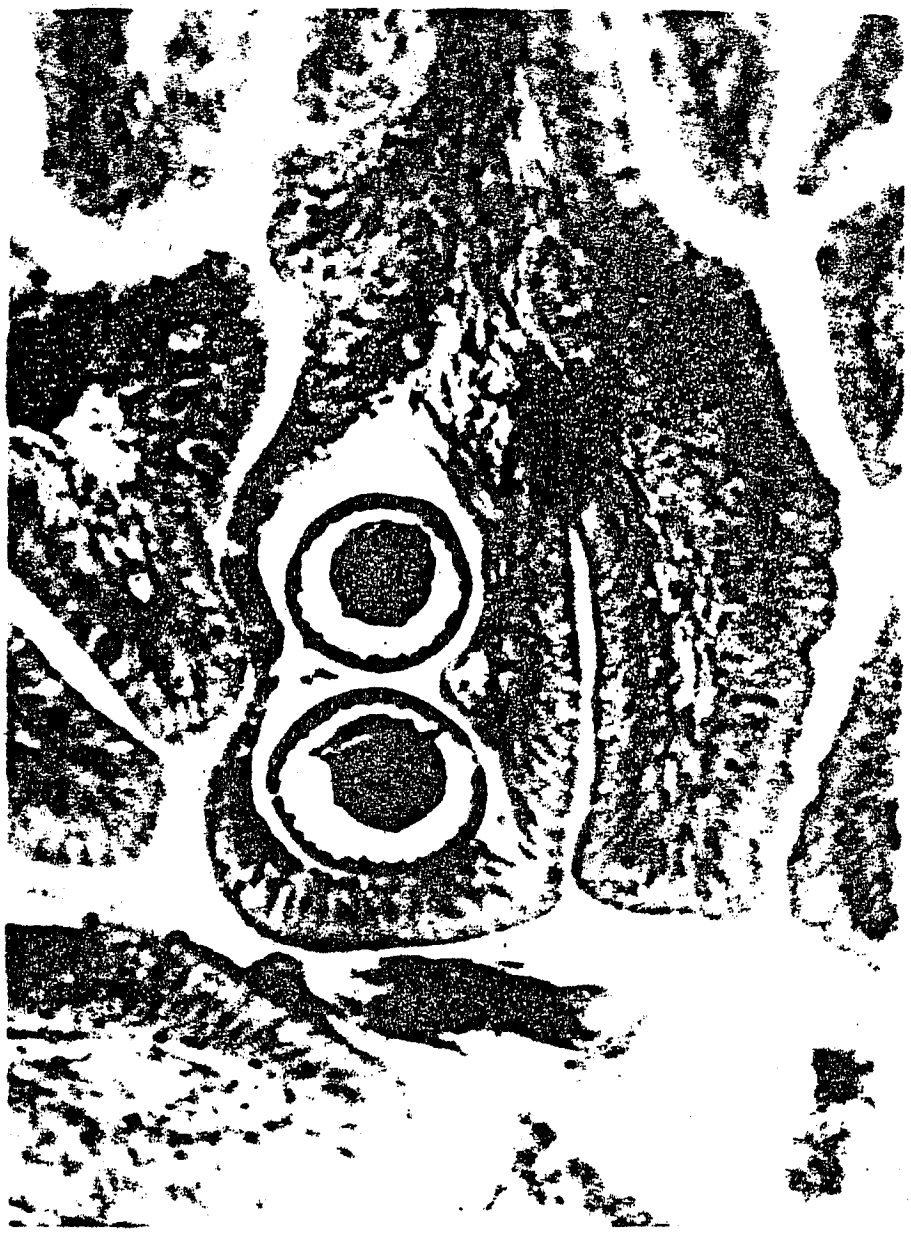

Figure 20. Photomicrograph of adult S. carolini in the submucosa of a bluegill. 130X.

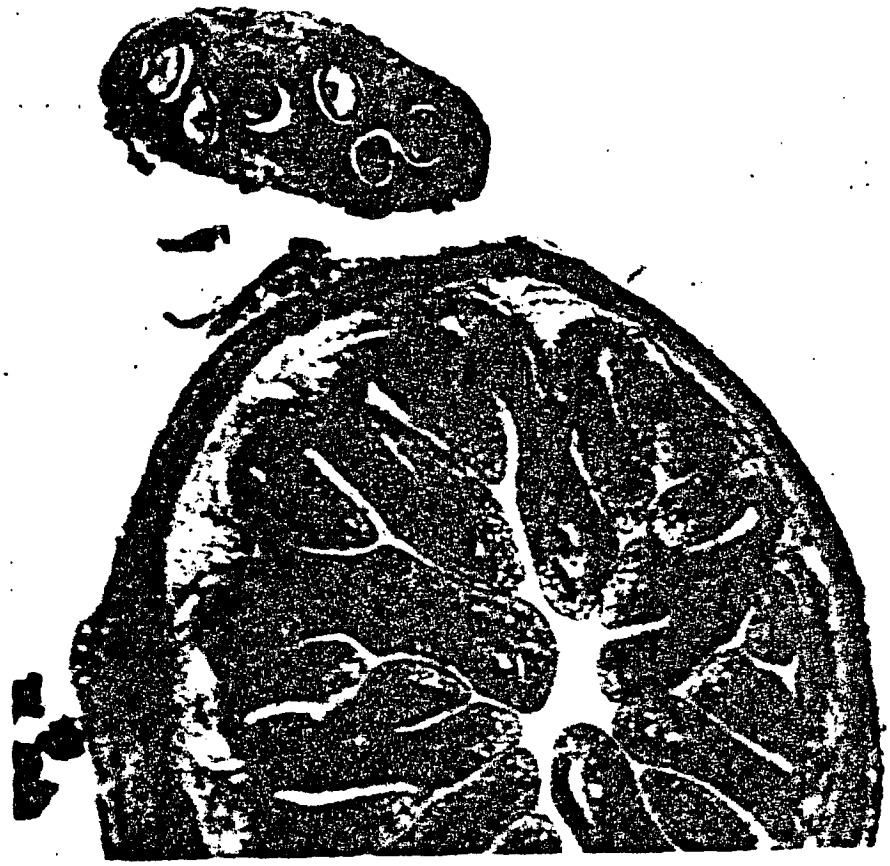

Figure 21. Photomicrograph of encapsulated adult *C. carolini* in the mesenteries surrounding the intestines of a bluegill. 40X.

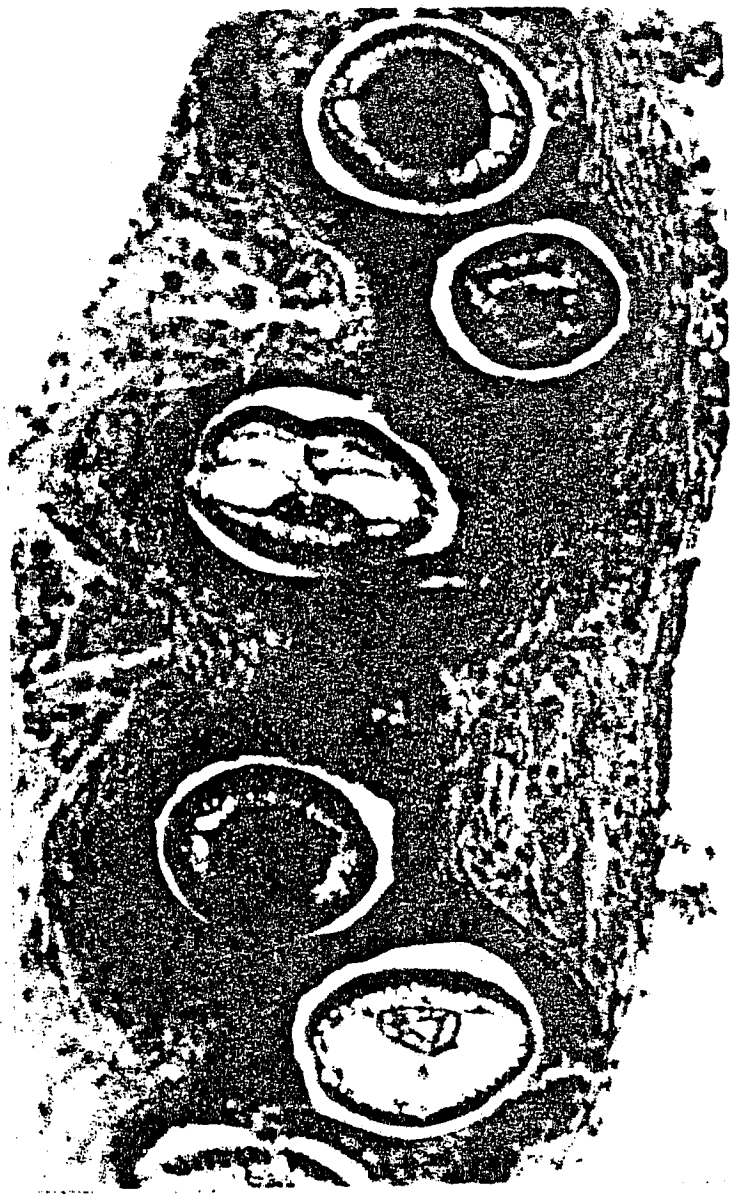

Figure 22. Photomicrograph of encapsulated adult S. carolini in the mesenteries surrounding the untestine of a bluegill. Note dark central fibrocytic area and outer fibroblastic area. 100X.

the last of which was never before observed.

Localization of Spinitectus was primarily in the anterior one-third of the intestine, an area where a large part of digestion and absorption of important constituents of the diet transpires. Therefore, damage created to any portion of the intestinal wall may result in malabsorption. Malabsorption, however, is extremely difficult to determine, since the functional reserve of the intestine is large. So a disturbance in one section of the intestine may be compensated for in the organ as a whole.

POPULATION BIOLOGY

There was an indication of change in the prevalence in Spinitectus over the past 50 years. The prevalence of S. carolini (Table IV) decreased in the following fish: rock bass, pumpkinseeds, bluegills, smallmouth bass, largemouth bass, white bass, and yellow perch. An increase in the prevalence of S. carolini was observed in catfish and bullheads, however, these increases were slight (Table IV).

The prevalence of S. gracilis (Table IV) exhibited opposite trends as it increased in the following fish: rock bass, pumpkinseeds, bluegills, smallmouth bass, largemouth bass, white bass, and yellow perch. Decreases in the prevalence of S. gracilis were observed in catfish and crappies (Table IV).

The prevalence of S. carolini was highest in rock bass (79%), while the prevalence of S. gracilis was highest in smallmouth bass (39%) (Tables IV & V). The first indication of dual parasitism by S. carolini and S. gracilis was observed in the following fish: rock bass, yellow bullheads, channel catfish, pumpkinseeds, bluegills, smallmouth bass, and largemouth bass (Tables IV & V). The only fish found to be void of Spinitectus infections were the black and white crappies.

Spinitectus infections increase from May through

September in rock bass, and from May through August for smallmouth bass and bluegills (Figs. 23, 24, 25, & 26). The mean percent infection was greatest in younger rock bass (Fig. 23), young of the year fish through age class II. Infection rates dropped in age class III and older rock bass from 90% to 50% (Fig. 23). Smallmouth bass, however, showed low mean percentages of infection in young of the year fish through age class II (Fig. 24). Older smallmouth bass, age classes III and greater exhibited higher mean percentages of infection (Fig. 24).

Table VI showed that smaller fish were more heavily infected with Spinitectus, and that infection rates declined from 94% to 63%. It should be noted though that as the infection rates declined in larger rock bass, there was a concommitant rise in mean worm burdens from 8 to 22 (Table VI).

The mean worm burdens increased from May through August followed by a rapid decline (Figs. 27 & 28). These trends were observed during both collecting seasons (1978-1979) (Fig. 27). Female/male ratios approached an average of 2:1 (Fig. 28).

Recruitment of third stage larvae of S. carolini and S. gracilis occurred from June through September (Fig. 29). Third and fourth stage larvae were both recovered from the fish throughout the months of June through September, however, the number of fourth stage larvae usually

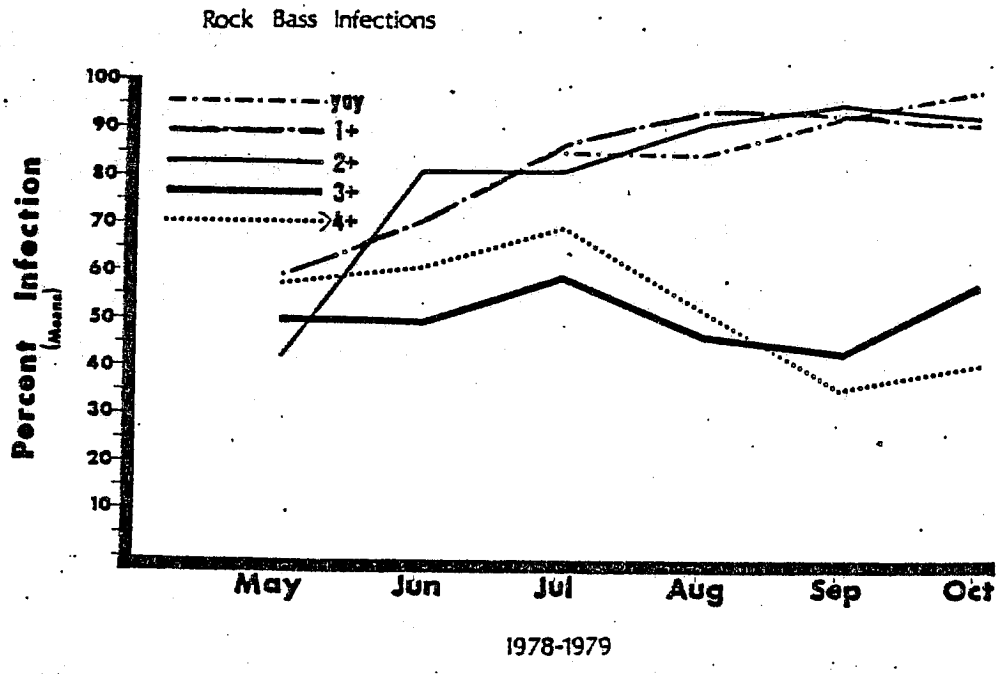

Figure 23. Percent infection of Spinitectus in rock bass by age class (1978-1979). yoy-young of year fish.

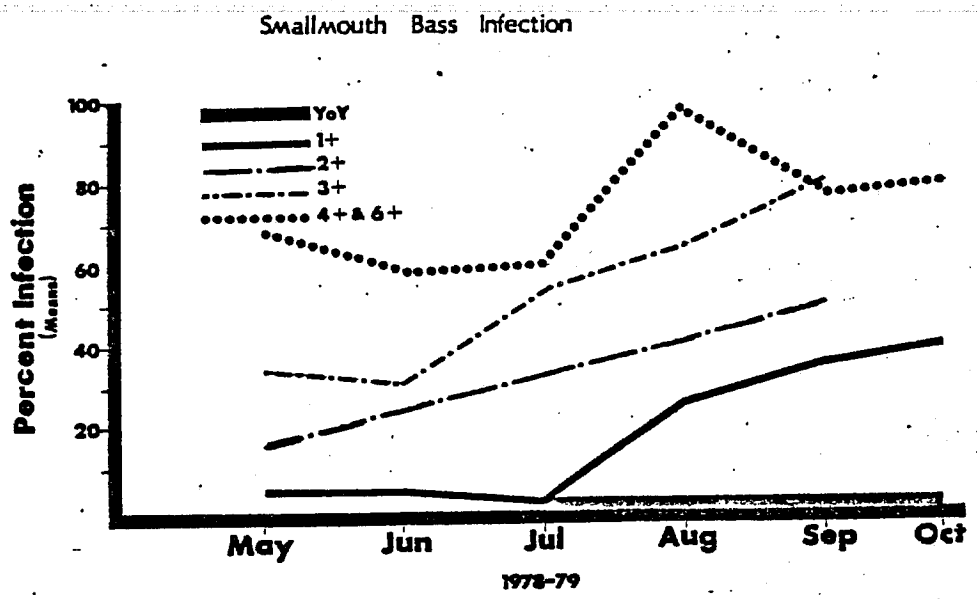

Figure 24. Percent infection of Spinitectus in smallmouth bass by age class (1978-1979). yoy-young of year fish.

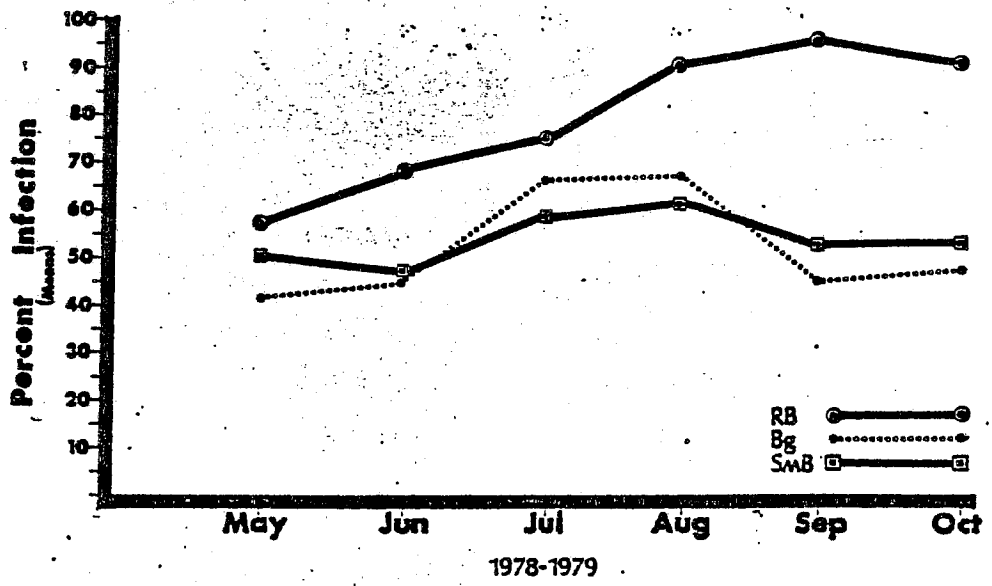

Figure 25. Percent infection of rockbass (RB), bluegills (Bg), and smallmouth bass (SmB) with Spinitectus (1978-1979).

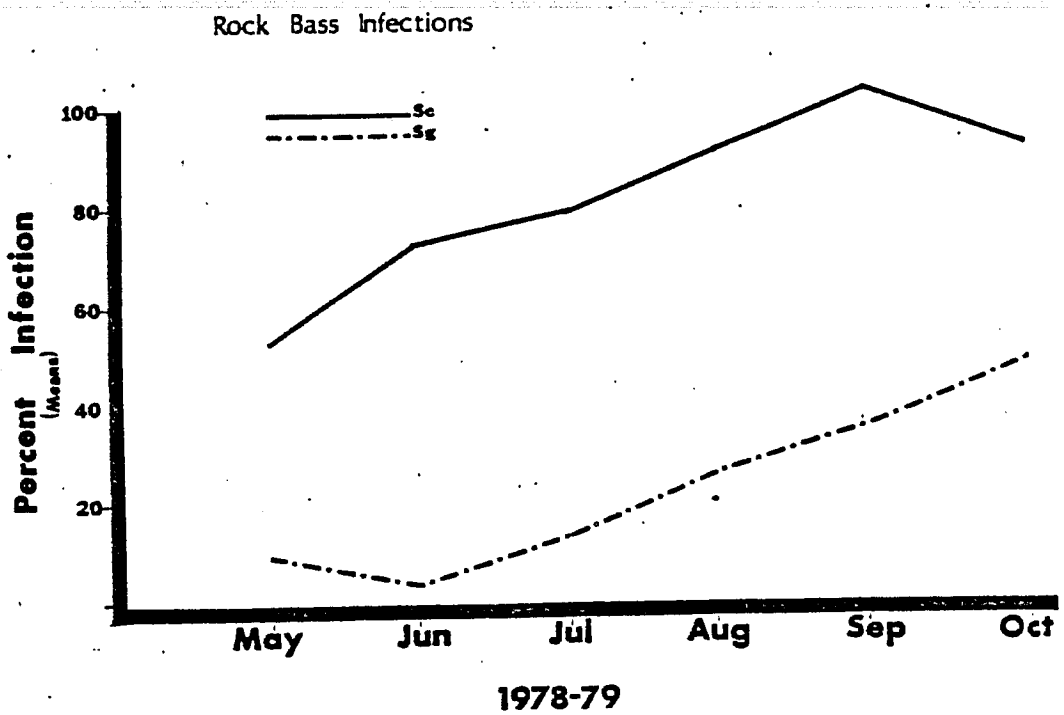

Figure 26. Percent infection of rock bass with Spinitectus carolini (Sc) and Spinitectus gracilis (Sg).

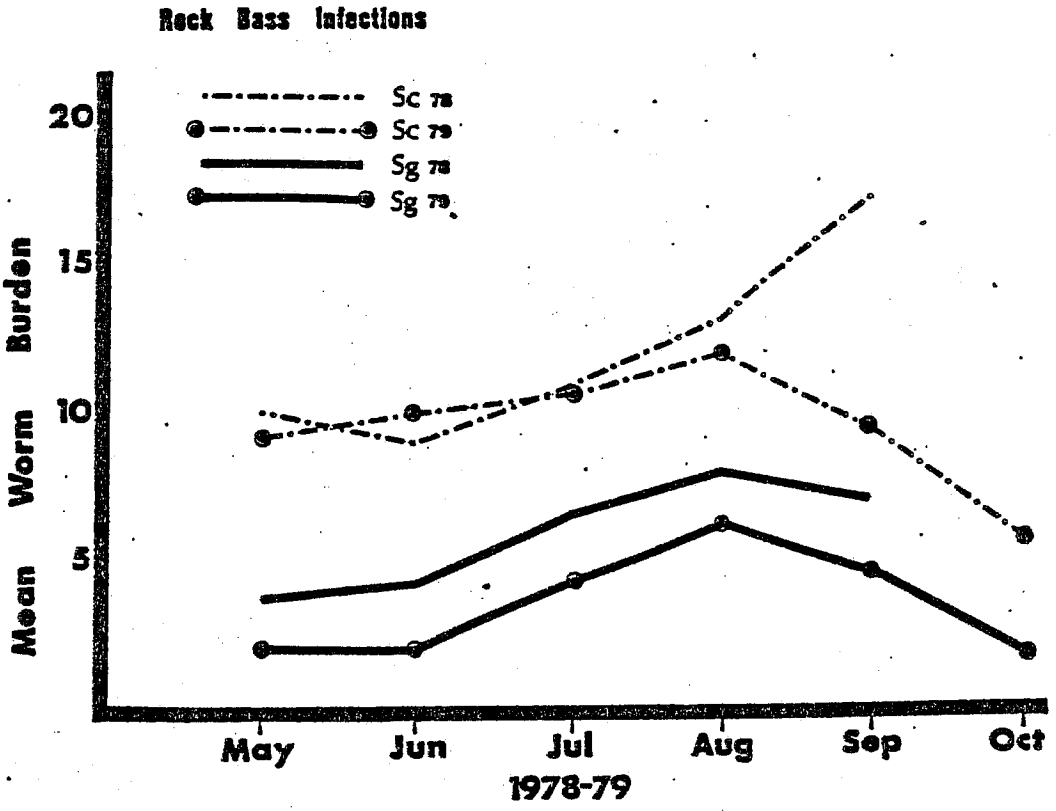

Figure 27. Mean worm burdens of Spinitectus carolini (Sc) and Spinitectus gracilis (Sg) in rock bass (1978-1979).

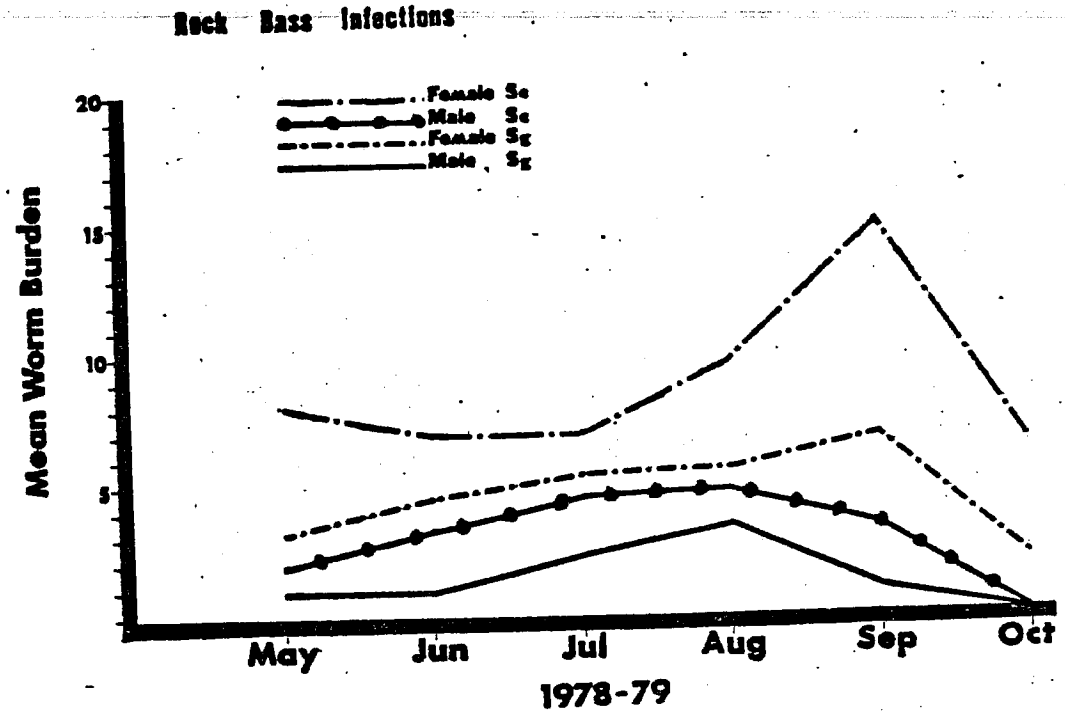

Figure 28. Mean worm burdens of Spinitectus carolini (Sc) and Spinitectus gracilis (Sg) in rock bass (1978-1979), according to male and female Sc and Sg.

Rock Bass Infected with Spinitectus sp.

Length	# Examined	# Infected	% Infected	Mean Worm Burden
<100	107	100	94	8
101-150	117	105	90	10
151-200	112	100	89	14
201-250	129	71	55	19
>251	68	43	63	22

Table VI. Rock bass infected with Spinitectus, S. carolini or S. gracilis (1978-1979). Length in millemeters. All rock bass collected from the western basin of Lake Erie.

outnumbered the number of third stage larvae (Fig. 29).

An examination of the growth (length/weight) of rock bass infected with S. carolini or S. gracilis versus rock bass uninfected with these 2 nematodes had shown an inhibition of growth. Initially (age classes 1 & 2) there was an accelerated growth (Figs. 30 & 31) of uninfected rock bass, however, after age class II there was a decline in the growth of infected fish (Figs. 30 & 31).

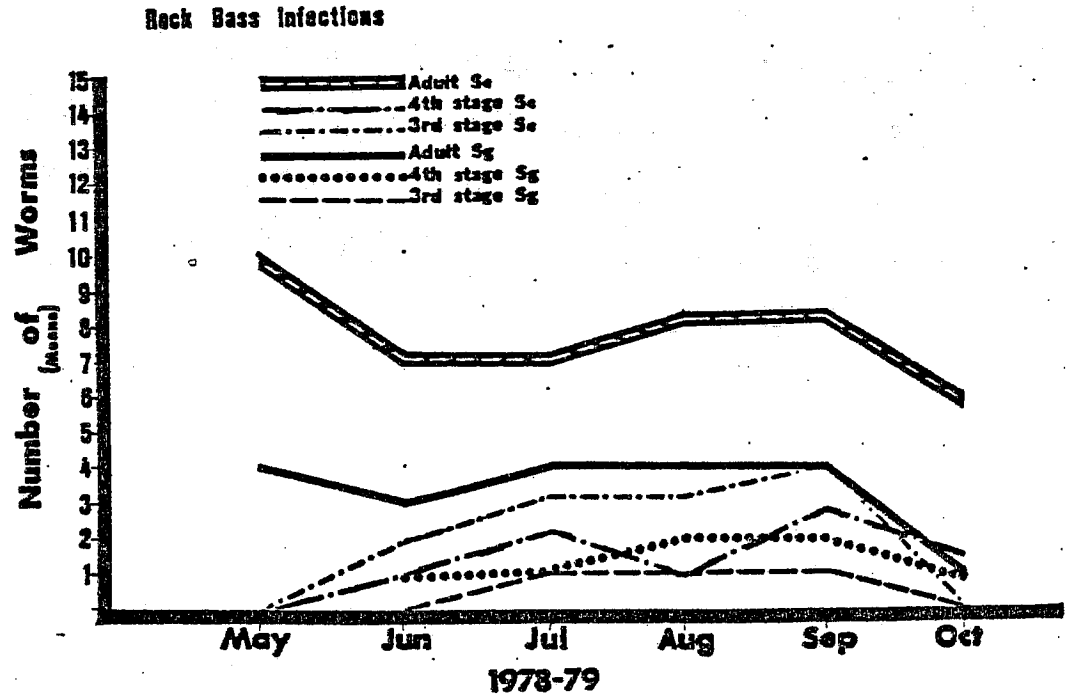

Figure 29. Mean worm burdens of Spinitectus carolini (Sc) and Spinitectus gracilis (Sg) in rock bass (1978-1979) by stages of development, larval stages (3rd & 4th) and adults.

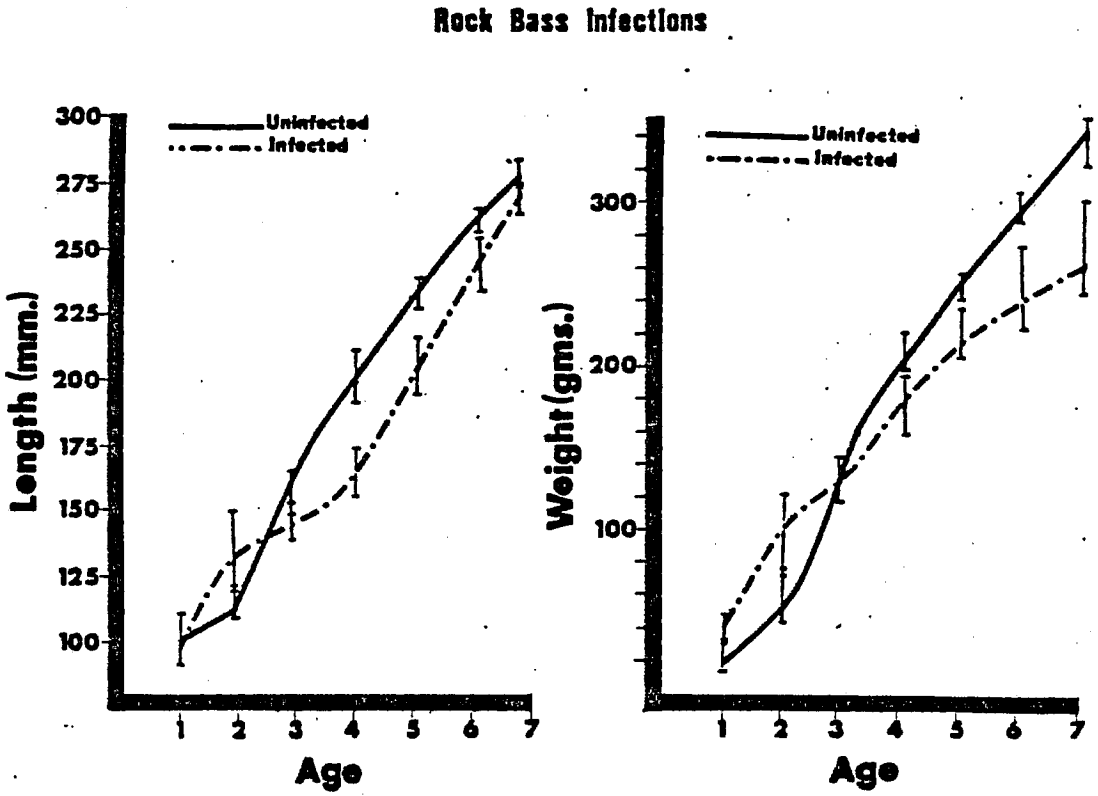

Figure 30. Length/age and weight/age relationships of uninfected rock bass and rock bass infected with Spinitectus. Bars represent 95% confidence intervals.

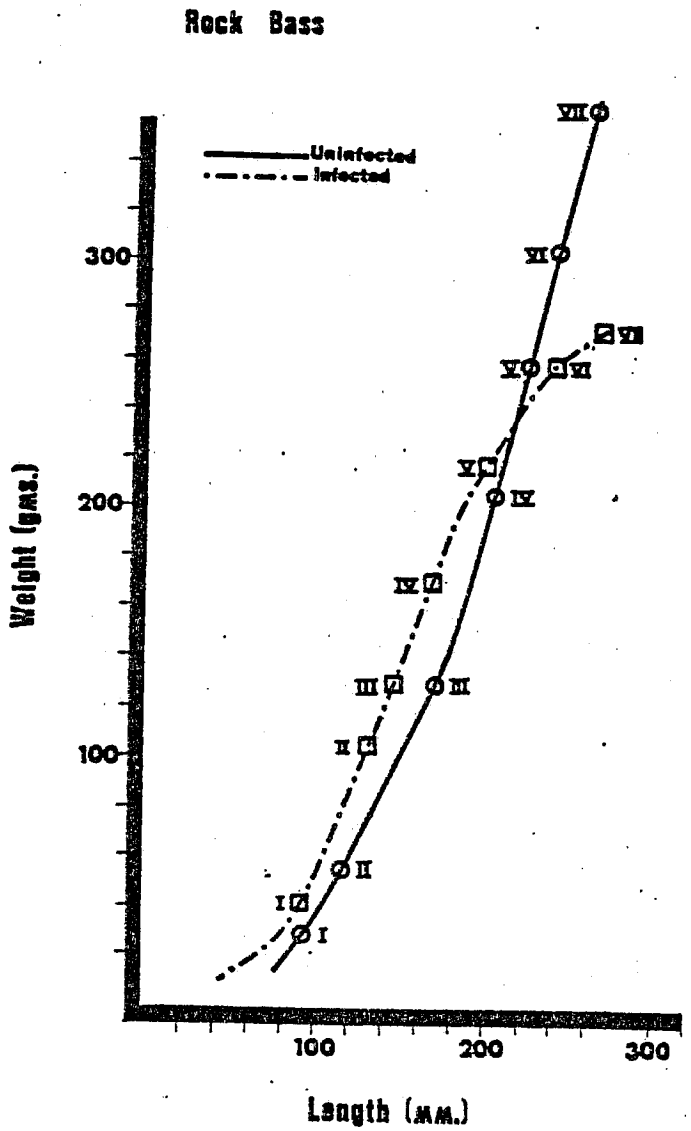

Figure 31. Length/weight/age relationships of uninfected rock bass and rock bass infected with Spinitectus. Roman numerals represent age classes of the rock bass.

DISCUSSION

Typically the nematode life cycle consists of six stages: an egg, four larval, or more correctly, because there is no metamorphosis, juvenile stages, and the adult. Such is the case with Spinitectus carolini and Spinitectus gracilis.

Male and female nematodes occurred in the intestine of the fish definitive host. Copulation took place, and oviparous females layed eggs which passed with the feces. The eggs settled to the bottom where they were eaten by numerous larval aquatic insects (mayfly naiads, dragonfly nymphs, stonefly larvae, midge larvae, and collembolans). The larvated eggs hatched within the insects midgut which resulted in the relaese of the first stage larva. The first stage larvae penetracted the gut wall and entered the hemocoel within 6 hours post-exposure. The insects hemocytes repaired the serosa damaged by penetrating larvae. The first molt began 18 hours post-infection within the hemocoel and terminated by hour 36 which yielded a second stage larva. The second stage larvae grew and developed within the hemocoel for 5 days, after which they penetrated the abdominal muscles of the intermediate host tissues. Often hosts reacted to the presence of parasites attempting to phagocytise or encapsulate them, and many para-

sites were surrounded by a membrane or capsule of host cell origin. So the sheathed capsule that surrounded S. carolini and S. gracilis was not unique among parasitic infections. While this sealed off the Spinitectus larvae it did not kill them. The second molt to the third stage larvae occurred 8 days post-infection, within the capsule. The third stage larvae persisted in the capsule. They developed for an additional 8 days at which time they were infective for the definitive host.

Spinitectus larvae produced extensive damage to the abdominal musculature of their intermediate hosts. Similar results were found in each of the different groups of intermediate hosts studied. Spinitectus carolini and S. gracilis larvae, therefore, may play a role in the population dynamics of insect larvae, especially mayfly naiads and dragonfly nymphs, by limiting the number of individuals that will metamorphose to adults. Incapacitation of larval mayflies and dragonflies would also serve to facilitate the perpetuation of the life cycle of S. carolini and S. gracilis by increasing their availability to fish definitive hosts (Jilek and Crites, 1980a).

The effects of developing larvae on the nutritional state of their hosts can be substantial. Consequently, an infected mayfly would have to eat more food and ventilate more vigorously if it is to supply the parasites needs and mainatin the normal glucose levels of the hemolymph.

Even though the mere inclusion of an intermediate host in the life cycle may increase the probability of the parasite locating the definitive host, the activities of the infected hosts themselves may increase the likelihood of their being acquired by the definitive hosts. "Parasites have modified host behavior in relation to several different predation strategies and thus increased the vulnerability of infected host predation" (Holmes and Bethel, 1972). Through the pathology created by Spinitectus which may inhibit metamorphosis and the increased foraging and ventilating of the mayflies, it becomes evident that these infected intermediate hosts are subject to greater predation by fish definitive hosts.

In a stable ecosystem the factors resopnsible for determining specificity are generally themselves also stable, and specificity is strictly maintained. If however, the ecosystem is altered naturally or as a result of human intervention, specificity may also alter. Parasites may establish new systems if brought into contact with new hosts, or the changes may favor its establishment in a host which was previously regarded as an occassional one. The new host would have similar ecological requirements and therefore serve to perpetuate the species. Such may be the case in the life cycles of S. carolini and S. gracilis in Lake Erie during the 1950's when the mayfly populations

were all but eradicated. Thus other organisms such as chironomids may have filled the void in the life cycle, since natural occurring infections of S. carolini were found in chironomid larvae inhabiting Terwilliger's Pond.

After ingestion of an infected intermediate host by a fish definitive host the infective third stage larvae were freed from their capsules in the in the lumen of the fish's intestine. The third molt occurred 2 days post-infection of the fish. This molt occurred in one of three distinct microhabitats: the intestinal lumen, the submucosa, or the mesenteries surrounding the intestine. The fourth stage larvae grew and underwent differentiation of the sexes. The fourth molt occurred 14 days post-infection of the fish definitive host. Further growth and development of the adult continued for 7 days, after which they were sexually reproductive individuals.

The Pathology produced by S. carolini or S. gracilis was quite variable, and depended on the degree of migration exhibited by the fish parasitic third stage larvae. The third stage larvae were observed in three specific intestinal microhabitats (Jilek and Crites, 1980b). First, and by far the most common, was that the larvae remained in the intestinal lumen in close apposition to the mucosa, but not penetrating. Pathology here was minimal and was evidenced primarily by sloughing of mucosal cells.

Natural sloughing of mucosal cells was common, but may be accelerated by the nematode's movement and the spiny surface of the worm. Second, the third stage larvae penetrated through the mucosa and into the lamina propria and submucosa. Damage under these conditions was extensive when the larvae migrated to any appreciable extent. Third and the least frequent, the third stage larvae penetrated through the mucosa, submucosa, muscularis externa, and serosa and became encapsulated in the mesenteries surrounding the intestine (Jilek and Crites, 1980b).

According to Margolis "It has oft been speculated that parasites which penetrate the gut wall to invade body tissues may carry with them, or provide routes of entry for, pathogenic microbes". Therefore, S. carolini and S. gracilis may not only produce mechanical damage, but alsomay open the intestine and body cavity up to secondary infections.

Spinitectus carolini and S. gracilis have the capacity to penetrate through the entire intestinal wall, and though not observed in this study, may enter into other visceral organs, rather than being encapsulated in the mesenteries surrounding the intestine.

Experimental infections have shown that adult S. carolini ans S. gracilis could be transferred from one fish host to another by gavage. It is therefore highly probable that these encapsulations may serve as transfer

mechanisms for the infections of predatory fishes (Jilek and Crites, 1980b).

In general, the level of infection in any host depends upon the number of parasites infecting the host and the responses of the host to the infection such as: changes in recruitment and mortality rates. The older a host is, the longer it has had to make contact with a parasite. Change in age, however, is often accompanied by a change in structure, behavior or diet, which may change the probability of infection. From the foregoing then, it is postulated that a combination of factors are involved in Spinitectus infections. The diets of young fish differ from their older counterparts, so we saw a higher incidence of infection in younger fish (Fig. 23). The older fish, however, show higher worm burdens (Fig. 23), possibly indicating that younger fish cannot support heavy infections of Spinitectus. This may be due in part if not totally by the degree of pathology created by the parasites. Figures 30 and 31 show that as infected fish get older they exhibited substantial losses in weight and failed to grow as long as uninfected fish. Spinitectus carolini and S. gracilis may have a deleterious effect upon their hosts, which is unable to respond effectively against them. The parasites are able to survive in their host throughout the hosts life span, with an increase in worm burdens as the fish age. The effects of the parasite become progressively more evident, and older and

more heavily infected hosts may die-off as a direct result of the infection.

Infections generally commenced as soon as the ecological conditions permited contact between the host and parasite. The acquiring of new parasites or recruitment began in June (Figs. 23, 24, 25, 26, 27, & 28) as infective third stage larvae were made available to the definitive hosts. The availability was seasonal due to the life history of the intermediate hosts. It should be noted that the production of a new generation of infective third stage larvae occurred in less than one month, therefore, accumulation and persistance of later larval stages in an intermediate host may enable infective larvae to be available to fish all through the year. The time sequence of larval metamorphosis to adult insects appeared to be the time sequence for infection of the fish definitive hosts. Thus the percent infection, which is highest during the summer months (Figs. 23, 24, & 25) is dependent upon the increase feeding of fish during the warmer months and the availability of infective larvae, both of which follow seasonal patterns.

Spinitectus infections are probably not influenced by immune responses, since fish do not appear capable of mounting effective immune responses against their parasites, through the utilization of circulating or cell bound antibodies. The apparent inability of fish to respond immunologically to their parasites may well be a

reflection of the evolutionary state of development of fish immune responses in general. Furthermore fish are capable of producing at most only 2 circulating antibodies IgM and IgG. Even the production of these antibodies is temperature dependent. Fish may not exhibit an immune response, but it has been found that older fish have more goblet cells than do younger fish. The mucus contained in these cells has an inhibitory effect on some parasites. So, the infections of Spinitectus in older fish may in some cases be subject to the influence of goblet cell secretions.

No parasite lacks host specificity. Problems of host specificity involve physiologic factors, and they can best be solved by the use of experimental methods in life history studies. This host specificity is a function of physiologic specialization and evolutionary age. The specificity in Spinitectus infections, however, may be a matter primarily of ecology and behavior, than it is of physiology.

Broadly speaking, parasitic worms with direct life cycles are more host specific than are worms with an indirect cycle. On the other hand, worms that have an indirect life history generally exhibit more specificity for their intermediate hosts than for their final hosts, possibly because parasites are better adapted to their intermediate hosts, with which they have been associated for a

longer period of time. This appeared to be the case with Spinitectus infections. Spinitectus was recovered from numerous fish hosts which belonged to numerous widely separated groups of fish, whereas, the intermediate host specificity was restricted to larval aquatic insects. In a study of patterns of evolution in nematodes, Inglis concluded that, "In general parasitic nematodes are not host specific although they tend to be restricted to animals with similar feeding and ecological habitats". From the prevalence table (Tables IV & V) we can see that S. carolini and S. gracilis infected a considerable number and a wide variety of Lake Erie fishes. Other fishes may also carry Spinitectus infections, however, they were not examined. It is believed that S. carolini and S. gracilis exhibit little host specificity for their definitive hosts.

Successful transfers of third stage, fourth stage and adult S. carolini and S. gracilis from one fish to another has provided a second means by which definitive hosts may become infected. The first and probably most frequent method would be the usual life cycle course involving the aquatic larval insect intermediate host and the fish definitive host. The second pathway would involve the insect intermediate host, a fish transport host, and the final fish definitive host (Fig. 32). According to Jilek and Crites (1980b), the mesenteric en-

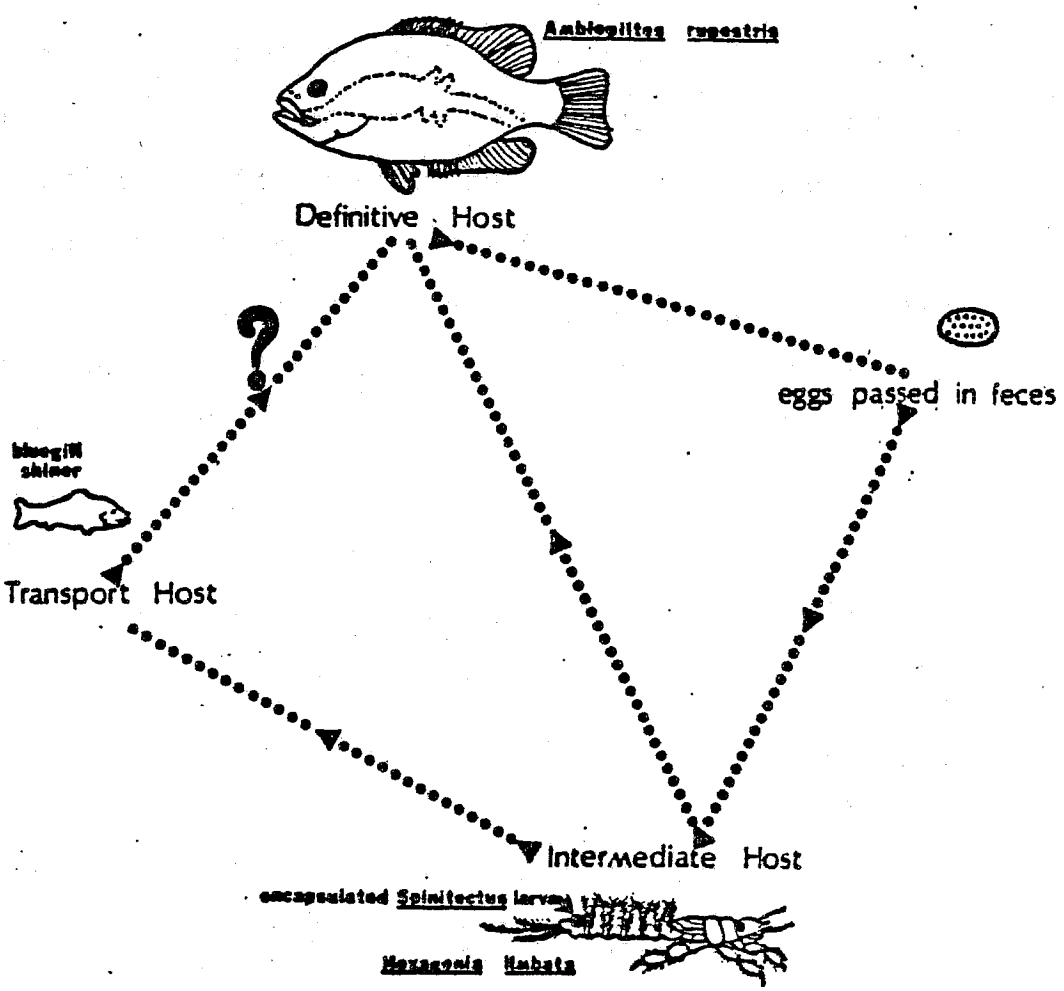

Figure 32. Diagram of the life cycle of *S. carolini* or *S. gracilis*.

capsulations may function as a transfer mechanism, whereby, one host would become infected by feeding on another host, or for that matter enteric S. carolini and S. gracilis may transfer should one definitive host be eaten by another.

Prior studies by Jilek and Wolff (1978) and Holl (1932) indicated the host specificity of S. gracilis and S. carolini may be very broad, and cover several classes of vertebrates.

Studies by Bangham and Hunter (1939) and Bangham (1972) indicated an absence or low prevalence of S. carolini and S. gracilis in Lake Erie's important game fishes. By comparing these surveys with the present study several differences were encountered. These differences were as follows: 1) the first indication of dual parasitism by S. carolini and S. gracilis; 2) an indication of increase in the prevalence of S. gracilis; 3) an indication of an overall decrease in the prevalence of S. carolini; 4) new host records for S. carolini and S. gracilis in Lake Erie fishes sampled (Jilek and Crites, 1980c). Dechitiar (1972) did not report any new records of Spinitectus in his survey of Lake Erie fishes from Ontario.

In an aquatic ecosystem undergoing succession changes take place in the parasite fauna that relate to those taking place in the host fauna, and the parasites may be influenced by these same factors that are influencing succession. Thus the successional changes occur-

ring in Lake Erie may be creating changes in the infection levels of S. carolini and S. gracilis. We see changes in predator-prey relationships with successional changes. The parasite fauna is therefore directly influenced by the feeding of its host and the trophic level of the latter. The nature of the predator-prey relationships can therefore serve as a potential index for predicting the structure of the parasite fauna in any given aquatic ecosystem.

The present study differs significantly from that of Keppner's (1975). His life history studies on Spinitectus micracanthus utilized but one host, Hexagenia limbata, whereas the present studied utilized numerous aquatic insects as intermediate hosts. Also Keppner (1975) utilized but one fish definitive host, Lepomis macrochirus, whereas the present studied utilized two species of Lepomis, L. cyanellus and L. macrochirus; two species of Micropterus, M. dolomieui and M. salmoides; and Ambloplites rupestris. Several differences were observed in developmental times, location of stages within the intermediate and definitive hosts, and the number of molts, between this study and Keppner's (1975).

The following represents a condensed version of this study, along with the results of Keppner's study (given in parentheses) (Table VII).

Adult S. carolini and S. gracilis occur in the py-

S. carolini		S. gracilis
1st	Intermediate Host	
Intestine (0-6 hours)		(0-6 h)
Hemocoel (6 hours-36 hours)		(6 h-36 h)
2nd		
Hemocoel (36 hours-6 days)		(36 h-7 d)
Abdominal Muscle (6 days-8 days)		(7 d-8 d)
3rd		
Abdominal Muscle (8 days-14 days)		(8 d-15 d)
3rd (14-16 days)	Definitive Host	(15 d-18 d)
4th (16-30 days)		(18 d-31 d)
Adult (30 ▶)		(31 d ▶)
Intestinal Lumen		
Submucosa		
Mesenteries		

Table VII. Summary of Spinitectus carolini and Spinitectus gracilis life cycles. 1st-first stage larvae, 2nd-second stage larvae, 3rd-third stage larvae, 4th-fourth stage larvae, adults. h=hours; d=days

loric caeca and intestinal tract (intestine only) of fish definitive hosts. Gravid females released eggs containing first stage larvae, which were subsequently voided with the feces from the fish's digestive tract. Eggs were eaten by aquatic insects (H. limbata), and hatched within the midgut. The released first stage larvae penetrated the gut wall within 6 (12) hours post-infection, and entered the hemocoel (longitudinal muscle cells). The second stage larvae grew and developed within the hemocoel (longitudinal muscle cell) for 5 (9-13) days, after which they penetrated the longitudinal muscles, and became encapsulated. The second molt occurred 8 (19) days post-infection. The third stage larvae remained encapsulated. Development to the infective stage occurred 14 (30) days post-infection. Sexes cannot (can) be differentiated in third stage larvae.

Infective third stage larvae were force-fed to fish definitive hosts. The third stage larvae were released from their encapsulations within the intestine of the fish. The third stage larvae either remained in the intestinal lumen, penetrated into the submucosa, or penetrated through the entire intestine and became encapsulated in the mesenteries surrounding the intestines (remain in the intestinal lumen). The third molt occurred 2 (16) days post-infection. The fourth stage larvae could be sexually differentiated (third stage). The fourth stage larvae molted to adults 14 days post-infec-

tion (fourth molt not observed). Development to sexually reproductive adults occurred 21 (26) days post-infection.

Keppner (1975) did not observe the fourth molt, however, he mentioned that he may have missed the molt. He stated that the third molt could have occurred in the mayfly naiad, and the fourth molt in the fish. The present study has shown conclusively though that there were four total molts in both S. carolini ans S. gracilis, with two each occurring in the intermediate hosts and two each in the definitive hosts. The molts were identified by the characteristic sloughing of the old cuticle.

Scanning electron microscopy has facilitated the differentiation and identification of these two common species of Spinitectus. The criteria used for distinguishing and identifying the species of Spinitectus vary greatly depending on the authors. The present study has revealed previously undescribed sensory structures, deirids. The deirids were located along the lateral lines, between the first and second rows of spines. En face views have revealed anatomical differences in the papillae, amphids, pseudolips, and oral opening. These structures were previously undescribed for either S. carolini and S. gracilis, apparently because no one ever looked at the en face views

of these two particular nematodes or saw little importance in recording their anatomy. In looking at other species of Spinitectus it has become evident that en face views are of great taxonomic value and may prove essential in differentiating species. Lastly, and possibly the most important structures taxonomically, are the spines. There is considerable variation among the North American species in spine number, spine morphology, spine length, and spine pattern. These variations in spines were evidenced in each of the stages of development. This spine variability, however, was non-existant within a given species, thus no intraspecific variation. Scanning electron microscopy functions as a useful tool in differentiating and identifying species of Spinitectus, and may possibly aid in the reduction of some species to synonymy.

Attempts were made to differentiate S. carolini from S. gracilis as two distinct species by experimental methods rather than microscopic examination. Uninfected fish were force-fed by gavage fourth stage female S. carolini or S. gracilis, simultaneously with adult male Spinitectus of the other species. The worms were allowed to remain in the fish for a period of 3-4 weeks after which they were removed by necropsy from the fish. Upon reaching sexual maturity, the worms could have copulated. Had copulation transpired, fertilization and subsequent pro-

duction of viable eggs should not have occurred unless S. carolini and S. gracilis are members of the same species. Fertilization did not occur, which further substantiates S. carolini and S. gracilis to be valid species.

CONCLUSIONS

1). The life cycles of Spinitectus carolini and Spinitectus gracilis were elucidated. The life cycles were quite similar, with both utilizing immature aquatic insects as intermediate hosts, and fish as definitive hosts.

2). Development included two molts in the intermediate host and two molts in the definitive host. The fourth molt is common for nematodes, but had never been observed in a species of Spinitectus prior to this study.

3). Histopathology due to the presence of S. carolini and S. gracilis was substantial in the immature insects and may adversely affect metamorphosis. Pathology was observed primarily in the abdominal muscle tissues.

4). Histopathology in the fish definitive host varied upon the specific microhabitat in which the parasites were found. The three microhabitats were: the intestinal lumen, the submucosa, the mesenteries surrounding the intestine. The greatest damage was incurred when the nematodes penetrated through the entire intestinal wall. Previously, Spinitectus spp. were thought to be non-pathogenic inhabitants of the intestinal lumen.

5). Recruitment of third stage larvae occurrs during the summer months, apparently when the intermediate hosts

are most available to the fish definitive hosts.

6). Mean worm burden and percent infection were greater for S. carolini.

7). Spinitectus male to female ratios were approximately 2:1 throughout the infection period.

8). There were indications of an increase in the prevalence of S. gracilis, and a decrease of S. carolini in Lake Erie fishes over the last 50 years.

9). Dual parasitism (S. carolini and S. gracilis) was recorded for the first time.

10). New host records were recorded for both S. carolini and S. gracilis from Lake Erie fishes.

11). Examination of Spinitectus by scanning electron microscopy has revealed the presence of deirids, amphids, sensory papillae, and distinct spine patterns.

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