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IMPACT OF PARASITIC "RED WORMS" ON LAKE ERIE FISHES

THE OHIO STATE UNIVERSITY
CENTER FOR LAKE ERIE AREA RESEARCH
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PROJECT NO: F-48-R-1 PROJECT TITLE: Impact of parasitic "red womrs" on Lake Erie fishes

STUDY NO: I STUDY TITLE: Impact of *Philometra* sp. on Lake Erie fishes

PERIOD COVERED: June 1, 1972-May 31, 1973

I. SUMMARY

Small *Aplodinotus grunniens*, 0+ age class and 1+ age class, often exhibit a syndrome called "pop-eye" during June and July. This disease is caused by the presence of maturing female nematodes of *Philometra* sp. Mature, gravid female nematodes penetrate the conjunctiva and extend outside for approximately two-thirds of their length. In the water the uterus prolapses through the body wall, burst and releases 60,000 first-stage larvae. The portion of the female which remains in the eye becomes encapsulated with connective tissue. Encapsulations amy persist in the eye for as long as 4 years.

Cyclopoid copepods were utilized as experimental intermediate hosts and maintained at 20 and 25°C. Copepods ingest first-stage larvae which penetrate the intestinal wall and enter the haemecoel. At 25°C the first cuticular molt occurs 4 to 6 days post-infection and the second molt occurs 8 to 12 days post-infection. Morphology and development of these three larval stages is described.

Freshwater-drum were administered infected copepods via a stomach-tube. The infective larvae penetrate the gut wall 7 to 9 days after infection and were found in the fish body cavity 10 to 12 days

post-infection. Larvae remain in the body cavity 10 to 22 days post-infection and then migrate to the eyes. They can be found in the eyes by 32 days post-infection. Sexual differentiation occurs rapidly after reaching the eyes, males which were unknown for this species are briefly characterized. After copulation the males die and the females continue to develop.

Immature nematodes migrate to the eyes of naturally infected fish during September and October invading the tissues surrounding the eye socket, the sclera and rectus muscles. Fertilized females grow to a length of 3 cm, they overwinter in the eyes and intra-uterine development of embryos occurs from April to July; during these months the females increase in size and reach a length of 9 to 10 cm. This increase in size is accompanied by a reddening and inflamation of the eye and pressure causes the eye to swell and protrude from its socket.

Eighty-two percent of the naturally infected *Aplodinotus grunniens* examined were infected with some stage of *Philometra*. Small freshwaterdrum 5 to 20 cm in length, 0+, 1+ and 2+ age classes, harbour the greatest burden of living philometrids. Double eye infections are more prevalent than single eye infections.

The transmission cycle of *Philometra* sp. in *Aplodinotus grunniens* is an annual cycle. One generation occurs each year and it is seasonly timed and predictable.

II. CONTENTS

information on which year classes of fish hosts are most affected by these infections and if infected fish are more susceptible to predation. (3) It will demonstrate in which seasons of the year there is greatest loss of fish hosts to these disease agents. (4) It will show the affect on the quality of the fish now being caught by sports fishermen. (5) It will yield both quantitative and qualitative information as to the desirability and need for inspection of fish from western Lake Erie before they are transported to other sites for stocking or for sports fishing. (6) It will demonstrate the season of the year or year class of fish most propitious for transfer of Lake Erie fish infected with these parasites. (7) It will demonstrate the locations and times of parasite-intermediate host-fish host contact and show where controls may be applied. (8) The statistical, quantitative and biological data will make possible predictions of the affect of the parasites if or when Lake Erie becomes thermally enriched.

IV. OBJECTIVES

Three research objectives were involved in this study. 1) An Experimental Investigation: Determination of the transmission cycle of *Philometra* sp. Demonstration of what intermediate host are involved in the processes of transmission and how and when the infective stages are passed from definitive host to another. Demonstration of the sequence and times of development of the stages in different hosts involved. 2) A Descriptive Investigation: Description of the parasitic adult and developmental stages occurring in the different hosts

involved. Description and demonstration of routes of migration through the organs and tissues of the hosts and the resultant pathology. 3) A Population Investigation: Determination of the incidence and intensity of infection and the effects on natural populations of fish hosts in western Lake Erie.

V. PROCEDURES

Samples of freshwater-drum were taken from Lake Erie during the ice free periods of the year from the inception of this study, June through October 1972 and April and May of 1973. Twenty-eight samples were collected during this year from two sites: 1) from a seine-haul at Cold Creek on Sandusky Bay, Lake Erie; and 2) from open lake trawls taken with the moter vessel Bio-Lab between Green and Rattlesnake Islands in Lake Erie. Fish were iced immediately and brought to Stone Laboratory during the summer and fall months or to the Parasitology Laboratory at The Ohio State University, Columbus, Ohio in April and May. The fish were autopsied and the eyes, body cavities and viscera were examined, using a dissection microscope, for the presence of living philometrids and encapsulations of whole or partial worms. The data for each fish examined was recorded. Seven-hundred and ninety-nine *Aplodinotus grunniens* were visually autopsied, 42 were experimentally infected and 30 were examined by digestion techniques in the period June through November 1972. Eighty-five were autopsied during April and May 1973. The total number of fish examined this year was 956.

First-stage larvae were released from gravid, mature female philometrids by placing the entire female in distilled water or lake

water until the uterus prolapsed through the body wall. Free-living, first-stage larvae were tested for longevity at 20 and 25 degrees centigrade.

Cyclopoid copepods were collected with a plankton net and cultured at 22 to 26 degrees centigrade until needed for infection experiments. More than 3000 cyclopoid copepods were experimentally infected by exposing them to first-stage larvae in petri dishes, cyclopoid copepods readily ingest the larvae. Infected copepods were maintained in controlled temperature cabinets at 20 and 25 degrees centigrade. Experimentally infected copepods were examined daily, they were crushed under a cover slip to release the larval nematodes and the larvae were studied microscopically to determine the extent of development and molting. All developmental stages were photographed.

Living *Aplodinotus grunniens* and *Perca flavescens*, to be used in infection experiments were brought to the laboratory in iced, oxygenated water, they were placed in large tanks of lake water and allowed to stabilize. These fish which lived for more than two days, and showed no signs of disease or abnormal behavior were transferred to aquaria and maintained at ambient temperatures, not more than four fish were placed in an aquarium. Each aquarium was equipped with an air pump and filter system. All fish were fed only commercially grown earthworms.

Each fish used in infection experiments was anethetized by placing it in a solution of quinaldine (0.25 ml of quinaldine per 2.5 gallons of water). These fish were autopsied at pertinent intervals after infection to determine the migration and extent of development of the

larval nematodes in the fish host. The intestines of some experimentally infected fish were examined by digestion in artificial gastric solutions to determine the migration of larvae through these tissues. Parallel examinations of fish from natural populations were conducted to confirm that the same processes were occurring at the same time in fish in Lake Erie. During June and July sixteen heads of small "pop-eyed" sheepshead were excised immediately upon removal from the trawl while still on the Bio-Lab and fixed in alcoholic Bouin's solution for later sectioning. This process was repeated for "young of the year" freshwater-drum in September and October. These heads were later double-imbedded in celloidin and paraplast, sectioned and stained with Mallory's Triple stain and hematoxylin and eosin stains. Sections through the eyes of a normal, uninfected sheepshead were prepared for comparative purposes.

All nematode stages were studied alive utilizing both ordinary and phase microscopy. Nematodes from specific samples and of each stage of development were fixed with alcoholic Bouin's solution for later sectioning or Alcohol-Formalin Acetic Acid (AFA) solutions for preperation of whole mounts. These nematodes fixed with AFA were later cleared in glycerin-alcohol and mounted in pure glycerine on microscope slides for study, in some cases, nematode stages were stained with Semichen's Carmine stain before clearing to enhance the study of certain morphological features.

A computer card was punched for each freshwater-drum autopsied from June through September 1972 using coding for FORTRAN computer language. The OMNITAB, CROSS-TABS and specially developed programs in FORTRAN language were utilized for analysis of population data.

VI. FINDINGS

Descriptive and Experimental Investigations of *Philometra* sp. Only adult, gravid, female *Philometra* sp. were found in the eyes of naturally infected *Aplodinotus grunniens* during April, May of 1972 and 1973 and June, July and the first two weeks of August 1972. No males of this species were present at this time.

The females grow from a length of 3 to 4 cm in April to 9 to 11 cm in July. Concomitant intrauterine development of the embryos occurs during this same period of time. Table A shows the progress of intrauterine development as it occurred in 1972. There is some evidence that this development is occurring later in 1973. During April and May the embryos undergo cleavage stages and develop to blastula and gastrula stages. In June the embryos develop through tadpole and early veriform stages to become inactive coiled larvae maintained within an egg membrane which surrounds all of the embryonic stages. The coiled larvae become active during late June and July, break through the egg membrane and move actively within the lumen of the uterus and are now ready for release. The first-stage larvae from 1 cm of uterus were counted using a Sedgewick-rafter cell and we estimate that each mature, adult female contains at least 60,000+ larvae in her uterus.

Small infected *Aplodinotus grunniens* between 10 and 20 cm in length, 0+, 1+ and 2+ age classes began to show the "pop-eye" syndrome in late June of 1972 and freshwater-drum with syndrome were found in natural populations until the first week in August. All fish which we autopsied, had two or more adult philometrids in the eye exhibiting the syndrome.

TABLE A

TIMES OF INTRA-UTERINE DEVELOPMENT-1972.

Date	Stages of Inra-uterine Embryonic Development
May 2	zygote, 2 cell, 4 cell, 8 cell stage
May 16	4 cell stage through early blastula
May 30	blastula and early gastrula
April 19	blastula, late gastrula through early tadpole stage.
June 21	late gastrula thru early vermiform stages
June 26	vermiform and coiled larvae
June 28	coiled larvae and inactive first-stage larvae
June 29	coiled larvae through active first-stage larvae
July 5	coiled larvae through active first-stage larvae
July 11	coiled larvae through active first-stage larvae and release-streaming
July 18	active first-stage larvae-streaming
July 20	active first stage larvae-streaming
July 26	Active first-stage larvae-peak of streaming, encapsulations began

Either the right or the left or both eyes simultaneously may be infected. Exact data for this will be presented later in the report. An infection with a single worm may or may not cause the syndrome, usually it does not. The syndrome was photographed. As the adult females grew in size they filled the orbit of the eye and invaded the tissues surrounding the socket, the rectus muscles and the sclera. At this time pressure builds up within the orbit, the tissue of the eye is reddened and inflamed and the eye begins to swell and protrude from the socket. Heads of fish exhibiting the "pop-eye" syndrome were excised, fixed in different solutions and are in the process of being sectioned. Sections of the head of one uninfected fish have been completed and is being studied for normal anatomy and histology for comparative purposes.

The adult female penetrates the conjunctiva, usually at the margin, and extends into the water streaming posteriorly along the side of the fish. The uteri prolapses through the body wall of the nematode and thousands of active first-stage larvae are released into the water. The adult mature philometrids do not have a vulva, genital pore, at this time, although we have now demonstrated that one is present in an earlier period of 5th-stage development. We have prepared stained cross-sections of the adult gravid females and these demonstrate that the body wall is laterally very thin at the time prior to penetration and streaming, with muscles only in the dorsal and ventral areas. Microscopic examination of streaming females reveals that the uteri prolapse through weaker areas. We believe that prolapsis may be

caused by osmotic pressure. When mature, gravid females are carefully removed from the eyes and placed in physiological salt solution they seldom burst but when placed in distilled water prolapsis occurs rapidly. In 1972, the peak period of streaming from the eyes of *Aplodinotus grunniens* was between 20 July and 30 July. We have determined that *Philometra* sp. from eyes of freshwater-drum in Lake Erie has an annual cycle. The streaming of the mature, gravid females marks the end of one generation and larvae released represent the beginning of a new generation. A portion of the female becomes encapsulated by connective tissue. The encapsulations may exist in the eyes for several years and serve as markers of previous generations. These encapsulations have been photographed and some have been fixed for future sectioning and study.

Whether or not the small freshwater-drum are at least temporarily blinded is still an open question. We have removed as many as 9 mature worms from one eye and double eye infections are more prevalent than single eye infections (data on incidence and intensity of infection are presented later in this report). We are still preparing sections of celloidin imbedded eyes for study of the histopathology. Our studies of gross pathology show invasion of the rectus muscles and the sclera, inflamation, pressure great enough to make the eye swell and extend from the sockets and pressure on optic nerves. These conditions indicate that normal eye functions are greatly impaired. The recovery of encapsulations from 1+ age class and older fish does indicate that many freshwater-drum survive the infection if they are not lost from

the population because of the complications of the infection or preyed upon by larger predacious fishes. It is not uncommon to find both mature females and encapsulations of previous infections in the same eye of 1+, 2+ and 3+ age class freshwater-drum. This would indicate that there is no great natural immunity to infection. One to ten worms have been removed from one eye and multiple infections may impair vision more severly than single worm infections.

First-stage larvae released by uterine prolapsis were quite active and move vigorously but they soon settle to substrate and continue moving or attach by their tails and wave rapidly back and forth. When females containing active first-stage larvae were removed from infected eyes they were placed in petri dishes of distilled water where prolapsis occurred. These larvae were used for experiments and for morphological observations. The free-living first-stage larvae were photographed alive and their morphology studied but they still require more critical morphological study in the future. The main morphological features observed were: no perceptible lips; an open lumen to the esophagus; an intestine with no perceptible lumen and a long tapering tail with an open cavity. The free-living first-stage larvae range in length from 900 to 980 microns. They are capable of attatching by their tails but no glands could be ascertained utilizing oil immersion, phase microscopy. These larvae would be a good subject for study by electron microscopy and we plan to do this as our time allows.

Active free-living, first stage larvae were taken immediately upon release from the parent female worms and studied for longevity at different temperatures. They were maintained in distilled water in constant temperature cabinets at 20 and 25°C which approximately brackets the summer temperature of Lake Erie. They remained active for 7 days at 20°C and all of them had ceased movement in 10 days, they remained active for 5 days at 25°C and had seased active movement in 7 days at this temperature. When larvae cease active movement they are not dead but lie coiled in the bottom of containers, they could be stimulated to move only by touch or by creating a current in their immediate vicinity. All larvae were beyond stimulation for movement in 14 days at 20°C and 18 days at 25°C and they were presumed to be dead. Larvae were utilized for infection experiments only during their active periods. Free-living, first-stage larvae placed in a refrigerator at 5°C ceased to move but regained their active movements when warmed to 20°C. First-stage larvae can be stored at 5°C for 16 days and still remain infective for the first intermediate host.

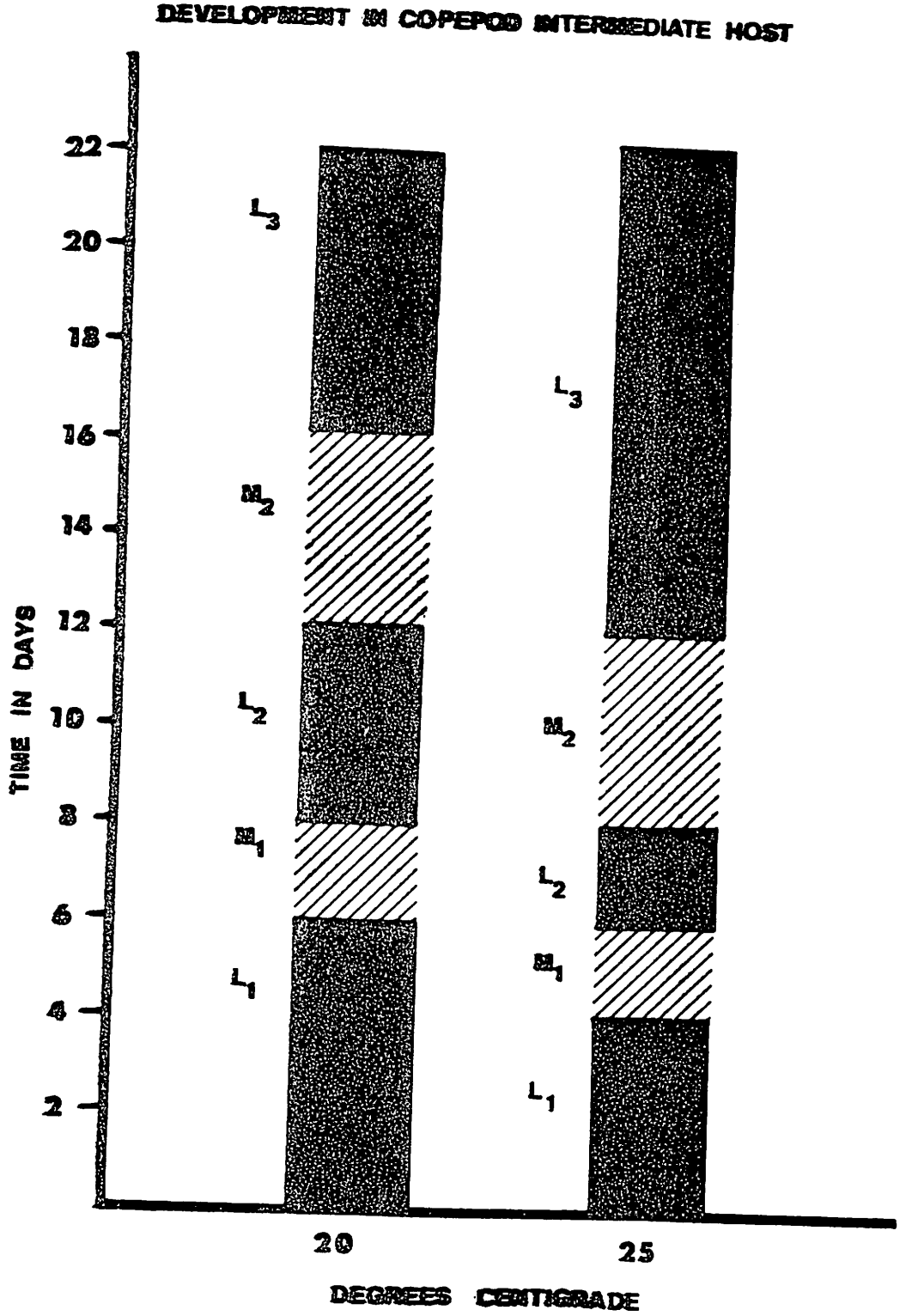
Because other nematodes of the Superfamily Doaceenculoidea and the Family Philometridae had been demonstrated to utilize cyclopoid copepods as intermediate hosts these crustaceans were chosen for experimental infections. Cyclopoid copepods were collected by plankton tow and cultured by feeding them *Paramecium*. the copepods were placed in small petri dishes with approximately 300 active, free-living, first-stage larvae. Ccyclopoid copepods readily ingest active, first-stage larvae. The ingested larvae penetrated the wall of the alimentary canal of the

copepod within 10 to 45 minutes and entered the haemocoel. More than 3000 copepods were infected in this manner during the summer of 1972 and used for further studies.

Two series of infected copepods were maintained, one at 20°C and one at 25°C. Infected copepods from each series were crushed on consecutive days and the parasitic larval stages examined microscopically for molting and development. Each of these was studied for morphological changes and photomicrographed. Each day at least one infected copepod from each series was fixed in Alcoholic Bouin's and placed in a vial for future study if needed. Parasitic first-stage larvae under go a development of the esophageal structures and a closure of the esohpageal lumen and the open area in the tail fills with tissue. The first-cuticular molt occurs 4 to 6 days after infection at 25°C and 6 to 8 days post-infection at 20°C (Graph A).

The larvae are now referred to as second-stage larvae. The second-stage larvae undergo a marked lengthing of the esophagus and development of intestinal cells. The second molt occurs 8 to 12 days after infection at 25°C and 12 to 16 days at 20°C. The second molt is always marked by anterior stomal structures in the molting cuticle. The larvae are referred to as third-stage after completing the second molt. The third-stage larvae remain alive for 20 or more days in the haemocoel and are infective for fish definitive hosts. A single copepod may harbour from 1 to 24 larvae in its haemocoel and survive but we tried to infect with only approximately 10 larvae because of the possibility of stress to the copepod host.

Graph A


DEVELOPMENT IN COPEPOD INTERMEDIATE HOST
TIME IN DAYS
22
20
18
16
14
12
10
8
6
4
2
L3
M2
L2
M1
L1
L3
M2
L2
M1
L1
20
25
DEGREES CENTIGRADE

Both living and preserved specimens of cyclopoid copepods infected during the summer were carefully studied and identified. Most prevalent among the infected copepods was *Cyclops vernalis* but *Cyclops bicuspidatus* and *Orthocyclops modestus* were also infected. Plankton samples taken weekly during the summer indicated that *C. vernalis* and *C. bicuspidatus* were the most common cyclopoid copepods in the Bass Island region of Laek Erie. These weekly plankton samples also enabled us to determine that free-living, first-stage larvae are released from streaming females *Philometra* sp. at the time when the copepod population was reaching its summer peak.

When possible infection experiments paralleled, as nearly as possible, the natural situation as it occurred in western Lake Erie. Copepods which were to be used for experimental infections of *Aplodinotus grunniens* were infected during the last two weeks of July. These copepods contained fully developed, infective third-stage larvae by mid-August. This same process was assumed to be taking place in the Lake at the same time. After 16 August 1972 no female *Philometra* sp. were found streaming from the conjuctivas of the eyes of naturally infected *Aplodinotus grunniens*. Only two partial living female nematodes were found in eyes after this date but many fresh encapsulations were present.

Experimental infections of freshwater-drum were begun on 1 August 1972. The fish utilized had been brought to the Laboratory during early July when they could not have fed upon naturally infected copepods containing fully developed, infective third-stage philometrid larvae. The

freshwater-drum, ranging in size from 15.3 to 19 cm in length, were placed in 15 and 20 gallon aquaria containing aged, filtered, areated well-water which had been treated with Nox-ich and Maracyn. The fish were allowed to adjust to the aquaria for two days and they were carefully observed for any sign of the "pop-eye" syndrome. Any fish which showed a suspected syndrome or streaming was eliminated from the lot. Only 42 fish were deemed satisfactory for experimental infections, even though larvae which might be found could not have come from any other source. Thirty-four of these fish survived long enough to yield experimental data. All fish were fed only fresh earthworms. The eleven fish which did not survive were autopsied as they died and none were infected.

Fish were infected as outlined in the procedures, each fish was administered four infected cyclopoid copepods containing four infective, third-stage philometrid larvae.

The results of these infection experiments were as follows:

1. Experimental fish examined 1, 2 and 3 days post-infection were negative. The larvae are extremely small at this time and it is probable that they were simply missed.

2. Experimental fish, 4 days post-infection. No larvae at visual autopsy. Alimentary canal and viscera fixed in alcoholic Bouin's for later sectioing. Sectioning revealed 3 nematod larvae in the intestinal lumen.

3. Experimental fish, 6 days post-infection. No larvae at visual autopsy. Alimentary canal fixed in alcoholic Bouin's for later sectioning. Sectioning revealed 2 nematode larvae in the intestinal lumen.

4. Experimental fish, 7 days post-infection. No larvae from visual autopsy. Five philometrid larvae from pepsin digest of the wall of the alimentary canal.

5. Experimental fish, 9 days post-infection. No larvae on visual autopsy. Nine philometrid larvae from pepsin digest of gut wall.

6. Experimental fish, 10 days post-infection. Two philometrid larvae in body cavity. One nematode larva in digest of the gut wall.

7. Experimental fish, 16 days post-infection. One larvae from the body cavity. No larvae from pepsin digest of gut wall.

8. Experimental fish, 18 days post-infection. Two larvae in the body cavity. No larvae from digestion of the gut wall.

9. Experimental fish 20 days post-infection. One larva in body cavity. No larvae in pepsin digest of gut wall.

10. Experimental fish 22 days post-infection. Two larvae in body cavity. No larvae in pepsin digest of gut wall.

11. Four placebo-control fish. Negative, no infection.

Three other experimentally infected fish were examined but were found completely negative. Presumably the infection was negative.

During August 1972 autopsies of freshwater-drum from natural populations began to yeild similar results. Two fish from an open-lake trawl of 28 August yeilded philometrid larvae from the body cavities. The alimentary of 10 other fish from this same trawl which had no larvae in the coelom were submitted to pepsin digest and 7 of the 10 had larvae in the gut wall. Two of 45 fish from the Cold Creek seine-haul examined on 5 September had philometrid larvae in their body cavities. These fish were 1+ and 2+ age class fish and both had encapsulations of previous

infections in the eyes. This indicates that reinfection does occur. After studying these results it was decided that emphasis should be placed on autopsy of "young of the year" or 0+ class freshwater-drum which were feeding on plankton and perhaps had ingested infected copepods. The next open-Lake trawl was taken 12 September 1972 and this trawl yeilded large numbers of "young of the year" freshwater-drum ranging from 3 to 10 cm in length (See Graphs 7 and 9). These fish yeilded many living philometrid larvae, 86.5% were infected, but to our surprise the larvae were not in the body cavity as expected but they had already migrated to the eyes.

These results keyed us again to examine our experimentally infected fish. The results were as follows:

1. Experimental fish, 32 days post-infection. Two larvae in the eyes, no larvae in body cavity.
2. Experimental fish, 34 days post-infection. One larvae in left eye. No larvae in body cavity.
3. Experimental fish, 35 days post-infection. Two larvae in eyes. No larvae in body cavity.
4. Experimental fish, 42 days post-infection. One larvae in right eye. No larvae in body cavity.
5. Placebo-control fish. Negative

Four other fish examined at this time were completely negative.

Migration to the eyes was rapid and unexpected, it occurs in a period of between 22 and 32 days after infection. This leaves a gap in our data which can be filled by carefully timed infection experiments during the summer of 1973. We must also determine the pathway of migration in both experimental and naturally infected fish.

Six *Perca flavescens* fed copepods containing third-stage infective larvae of *Philometra* sp. from the freshwater-drum were autopsied and all were negative for larvae of any type in the body cavities or eyes. These larvae are apparently uninfective for *Perca flavescens* which harbours a different species of philometrid in its body cavity.

The experiments demonstrate that when freshwater-drum ingest copepods containing mature third-stage larvae of *Philometra* sp., the larvae were released by digestion into the lumen of the alimentary canal. The larvae penetrate into the gut wall by 7 to 9 days after ingestion and occur free in films of liquid in the body cavity by 10 to 12 days post-infection. They remain in the body cavity until 22 days post-infection. Within a 10 day period the philometrid larvae migrate to the eyes of the infected fish and they can be found in the tissue surrounding the orbit, in the tissues of the rectus muscles and in the sclera of the eye by 32 days after infection.

The larvae removed from the body cavities of sheepshead during late August showed a development of gonads and lip structure but there was no sexually differentiation and there was no indication of further molting. The larvae ranged in length from 0.7 to 1.0 mm. Living specimens of these larvae were photomicrographed.

Trawls of 4 October and 24 October and a Cold Creek sample of 5 October 1972 provided many 0+ class freshwater-drum with many larvae in the eyes. Living specimens were photomicrographed but the majority were preserved for later study.

Preserved specimens were processed and arranged in sets according to the date recovered and in series according to length, from shortest to longest, and studied in that order. Careful study of living, photographed and sets of series of preserved specimens provided information concerning the development of these worms in the eyes of *Aplodinotus grunniens*. The worms ranged in length from 0.9 mm to 1.7 cm in the September samples and reached a length of 3.2 cm in the late October samples.

When the shorter nematodes were examined it was found that mature male specimens were present as well as females. Apparently sexual differentiation occurs as the worms migrate to the eyes or very quickly after reaching the eyes. Living and fixed male specimens were studied in detail. The males were mature, they had a fully developed testis, the vas deferens was packed with spermatozoa and they had well developed copulatory structures, two spicules and a gubernaculum. the spicules are used in copulation. The exact sex ratio has not been determined but it appears that there is one male per approximately 23 females.

Fifty-two species of *Philometra* have been described from fishes from different parts of the world, only three have known males. Five species have been described from freshwater fishes in North America and none of these have known males. One closely related genus, *Philometroides*, occurs in the skin and fins of suckers in the United States and this male was described in an unpublished doctoral dissertation at Colorado State University (M. Daley, Dissertation Abstrs. 67-5871, 1970). We have hesitated from the beginning of this project to give a species name to the *Philometra* which we have recovered from the eyes

of freshwater-drum in Lake Erie. Since we now have male specimens we feel that it can be correctly identified and described in detail.

The female specimens of each set were arranged according to their length from small to large and studied in that order as an ascending series. The females from the September sample sets varied from 2 mm to 11 mm in length and those from the October sample sets varied from 4 mm to 3 cm in length. This demonstrates that there is growth in length soon after the females enter the eyes. The smaller females have a well developed vulva (genital opening) with salient lips and a vaginal duct which makes contact with the vulva. As the gonad develops and becomes more apparent the vulva atrophies. Copulation of the male with female must take place while there is a functional vulva and vagina. This copulation must take place soon after the worms reach the eyes. An analysis indicates that 61 percent of the female specimens from September samples had a vulva or some trace of vulvular lips. In our October samples only 3.0 percent of the females had a vulva or some trace of vulvular lips. Thus the vulva atrophies as the female worm matures and this explains the absence of a vulva in mature, gravid females recovered later in the spring and summer months. These females when they stream from the eyes during summer have no other mechanism for release of larvae through the body wall except for the prolapsis of the uterus, there is no vulva.

There are other developments in the female reproductive system which were revealed by study of the series of sets of slides. These females definitely have two ovaries and two oviducts, one posterior and one

anterior. After atrophy of the vulva, the uterus becomes a single, sac-like tube estending most of the length of the body between the two oviducts. In the smaller worms studied the oocytes become observable and they appear to enlarge as they fill the lumen of the oviducts. As the females become progressively larger ova are released from the oviducts into the uterus which contains cells similar in size and shape to those found in the vas deferens of the male and these were presumed to be spermatozoa introduced at copulation. It is assumed, for the present, that fertilization occurs at this time. In the largest females of the October series the uterus was completely filled with zygotes. The females of this type are referred to as post-fertilization females. The largest of these females recovered in late October 1972 was 3.4 cm in length. Post-fertilization females recovered in April 1973 were only 3 to 4 cm in length. Apparently little growth occurs during the colder months of the year, November through March. Post-fertilization females taken in April had their uteri packed with zygotes none of these had started cleavage. The growth to the full length of 9 to 10 cm and the concomitant intra-uterine development of larvae occurs in the spring and early summer months, May through July, as described in the first part of this report.

We believe that males die soon after copulating with females and become encapsulated with connective tissue. Males were very scarce in the samples taken in late October. Very small encapsulations were found in the eyes of some freshwater-drum collected during the early

spring. Microdissection of 2 of these encapsulations revealed worms with spicules and gubernacula similar to those of living male specimens. Other small encapsulations contained worms which appear to be small female philometrids which did not survive the winter. Tissue sections of small encapsulations are being prepared to further confirm these observations. These encapsulations were taken from the eyes of 0+ age class fish.

Unrelated to the reproductive system the study of these ascending series also made other morphogenic developments and comparisons evident. Cephalic papillae begin to show development on the smaller specimens of the series, and they become quite evident in the larger specimens of the series. An examination of two en face preparations shows a distribution of papillae typical of the genus *Philometra,* 8 large papillae in the outer circle and 4 papillae in the inner circle with 2 lateral amphids.

Two caudal papillae so prominent in the fully grown, mature, gravid females begin their development at this time. These caudal papillae are visible both in lateral and dorsal-ventral view of the larger specimens examined.

The cells forming the cardia or esophageal-intestinal valve, become quite evident in smaller specimens of the series. Study of larger specimens shows that 4 very large cells comprise this organ at this stage of development.

A characteristic given for all nematodes belonging to the family Philometridae is that they supposedly have a single esophageal gland.

Actually this has been demonstrated in very few of the genera and species described. In almost all of the young specimens examined a very large gland is quite prominent in the tissue of the esophagus. We have also observed other smaller glands and many prominent nuclei utilizing phase contrast microscopy. Certainly the large gland is more prominent in this stage of development than it is in mature, gravid females. From a functional point of view one could hypothesize that this gland secretes the enzymes which enable these larvae and young females to migrate through tissues. In our future work this gland may merit a histochemical and microscopic study. If it does secrete such enzymes they may be related to tha pathology which occurs in the eye tissues of the fish host.

This year we have studied and photographed the morphological characteristics of the fully developed, gravid, females as they occurred in J July just before streaming from the eyes. Some investigators such as Rasheed (Rasheed, S. 1963, Journ. Helminthology, 37 (½): 89-130) have maintained that it is enough to describe and identify species of the nematode family Philometridae using only the characteristics of the mature, gravid females. We do not agree with this because as we have demonstrated there is a gradual development and in some species, such as that in the eyes of freshwater-drum, there is an annual transmission cycle where fully developed females occur only in one period of the year. Description and identification of Philometridae should also be based on male characteristics when ever possible.

In the course of our work for this project we have also recovered a philometrid nematode from the body cavity of *Perca flavescens*. We have identified this nematode as *Philometra cylindracea* Ward and Magath, 1916. This nematode was inadequately described and no males are yet known. Bangham first reported this nematode from yellow perch in Lake Erie in 1939 (Bangham, R. V. Studies on fish parasites of Lake Erie. Zoologica 24:385-448). We are convinced that even by Rasheeds criteria that the species in the eye of the sheepshead is not *P. cylindracea*. There are consistent differences in the adult females: the shape of the body; the shape of the head; the number of cephalic papillae; the shape of the tail; the shape, position and size of the caudal papillae; the shape and structure of the esophagus and cardium. In fection experiments using copepods infected with larvae from sheepshead were negative when these copepods were fed to 6 *Perca flavescens*. A study and comparison of larval and juvenile stages from the body cavity of *Perca flavescens* and the eyes of *Aplodinotus grunniens* made during the year further stregthens our belief and contention that these are two different species. The larval specimens from perch have characteristics which correspond with those of adult specimens from perch. Those from the eyes of freshwater drum have characteristics which correspond with the adults from the eyes of freshwater-drum. These comparisons of both larvae and adults will require more specimens, time and study, but we plan to describe and publish the species from the freshwater drum as new and we believe that the species from the perch is *Philometra cylindracea* but requires a redescription in published form.

Mr. Robert Ashmead, research associate on project F-48-R, has decided to study the transmission cycle and the population ecology of *P. cylindracea* as a thesis project for the degree Master of Science.

Population Investigations of *Philometra* sp. In our project proposal we made statements to the affect that the incidence and intensity of infection of parasites in different size and age classes of natural populations of fish hosts should be checked throughout the year when possible. We pointed out that such information is usually missing in studies dealing with parasites of fishes and that this type of data could be useful to fisheries biologists and in fisheries management. This section of the report presents quantitative data for infections of *Philometra* sp. in the eyes of freshwater-drum from Lake Erie. It presents the frequency distributions of freshwater-drum examined from June through October 1972 and the frequency distributions of the population of *Philometra* sp. infecting them. It also presents data concerning specific months of the year when infections occur and specific size and age classes of freshwater-drum infected.

The scatter-diagram graphs which are presented here were obtained in the following manner. A single computer data card was punched in FORTRAN computer language for each fish collected and autopsied from natural populations. Experimentally infected fish and fish examined by digestion techniques are not included; of more than 1000 fish examined 802 fit this category and are represented on each graph. Data was taken directly from research notes, Volumes I through III, Study I, Project

f-48-R-1, and punched on cards in the exact sequential order of examination after determing the exact program code to be used. The OMNITAB program was used to plot the scatter-diagrams listed below as graphs.

Graph 1. A copy of the computer read-out showing the frequency of mature, adult, gravid female worms removed rom the eyes and the lengths of the fish hosts from which they were removed.

Graph 2. A copy of the computer read-out showing the frequency of juvenile females in the eyes and the length of fish hosts in which they occur.

Graph 3. A copy of the computer read-out showing the frequency of encapsulations of spent adult females in the eyes and the length of fish in which they occur. Not included here are small encapsulations or encapsulations from the body cavity.

Graph 4. A copy of the computer read-out showing the frequency of mature, gravid, adult females in the eyes plotted against time, June through October (actual monthly periods in the same dimensions are shown on Graph 8).

Graph 5. A copy of the computer read-out showing the frequency of juvenile philometrids in the eyes plotted against time (actual monthly periods in the same dimensions are shown on Graph 8).

Graph 6. A copy of the computer read-out showing the frequency of encapsulations of spent, adult females in the eyes plotted against time (actual monthly periods in the same dimensions are shown on Graph 8).

Graph 7. A composite diagram composed of data from Graphs 1, 2 and 3, and drawn to the same dimensions. The frequency of adult females, juveniles and encapsulations removed from the eyes are plotted against the length of the fish from which they were removed. Uninfected fish are not shown.

Graph 8. A composite diagram composed of data from Graphs 3, 4 and 5, drawn to the same dimensions. The frequency of adult females, juveniles and encapsulations are plotted against the time when they were removed from the fish hosts. Uninfected fish are not shown.

Graph 9. This is a scatter-diagram of 835 fish autopsied (3 were too large and fell outside the dimensions set by the computer). The fish are shown exactly in the time sequence autopsied. Length is plotted against time collected and examined.

All graphs include fishes from both Open Lake Trawls taken between Green and Rattlesnake Islands, Lake Erie and the Cold Creek samples collected in Sandusky Bay.

Discussion, Length and Age Class of Freshwater-Drum Examined: Aging of sheepshead in Lake Erie presents some problems and we have accepted the measurements given by more expert investigators in recent works.

Russell Scholl and Carl Baker (personal communication) provided us with average length measurements of age classes of freshwater-drum taken at West Reef Lake Erie. Their data shows:

0	YOY	-to October- 16.12 cm ave.
1+	2 annuli	7.2 inches ave.=18.27 cm ave.
2+	3 annuli	8.6 inches ave.=21.82 cm ave.
3+	4 annuli	9.6 inches ave.=24.36 cm ave.
4+	5 annuli	10.4 inches ave.=26.40 cm ave.
5+	6 annuli	11.1 inches ave.=28.17 cm ave.
6+	7 annuli	12.0 inches ave.=30.46 cm ave.
7+	8 annuli	13.4 inches ave.=34.00 cm ave.

Tubb et al 1972 give the following data for freshwater-drum collected at Locust Point, Ohio, Lake Erie. (R. A. Tubb and associates 1972. Environmental evaluation of nuclear power plant. Fish, plankton and benthos populations prior to discharge. Ohio State University. Project F-41-R-4, Completion Report. 57 pp. As reported in C. E. Herdendorf and Elizabeth M. Hair 1972. Aquatic biology of Lake Erie in the vicinity of Locust Point, Ohio. CLEAR, Ohio State University. Report prepared for the Toledo Edison Company. See Table 8.)

Their data for freshwater-drum shows:

Age Class	Range in Cm	Mean Total Length in Cm
0+	3.8-14.2	$\bar{x}$- 9.9
1+	13.0-19.1	$\bar{x}$-16.3
2+	16.3-23.9	$\bar{x}$-21.6
3+	22.6-35.6	$\bar{x}$-29.7
4+	25.9-39.1	$\bar{x}$-31.2
5+	31.0-41.9	$\bar{x}$-37.1
6+	31.2-42.9	$\bar{x}$-38.4

Graph 9, which charts all fish examined by autopsy to date, shows the length distribution of these fish throughout the seasons when fish were collected in 1972. It appears that our samples may have been somewhat biased toward the 0+, 1+, 2+, and 3+ age classes. We stated in our monthly reports that we suspected some bias, but it is also true that longer and older age classes were not as abundant in our trawls and samples. Fortunately, as we will show below, and in future reports, the infections with living *Philometra* sp., both in incidence and intensicy, are most prevalent in age classes 0+ through 2+ regardless of the source of age class data.

The graph also demonstrates and confirms our statements in our August, 1972 monthly report that a new "young of the year" age class had entered our samples. This group stands out clearly in the lower right-had corner of the diagram. It is interesting that the read-out shows a distinct break in size between this "young of the year" class and the older fish collected. This break can also be seen on the left-hand side of the diagram for fishes collected in June and July. There is a break between fish plotted at 14.5 cm and 16.9 cm. The group of fishes below this break, taken during June and July, must certainly represent "young of the year" which developed the previous August,

September and October and have overwintered. This group is of particular interest as it is, as we shall demonstrate, most heavily infected with *Philometra* sp.

Frequency Distribution of Adult, Gravid Females: Graphs 1 and 4 are the computer read-outs for the frequency of adult females. These distributions are also plotted on composite graphs 7 and 8. Careful inspection of these diagrams shows that the majority of fish with infected eyes were collected in June and July, 1972, only 4 adult females were collected during August, 1972 and our research notes reveal that these were partially spent females or partially encapsulated (Graphs 4 and 8). The majority of infected fish collected measure in length from 9 to 14 cm, during June and July these would be 0+ age class which had just overwintered (Graphs 1, 7, and 9). It can be noted on Graphs 1 and 7 that there is a second but less intensely infected group of fish with less incidence of infection ranging in length from 16 to 20 cm; these would be 1+ and 2+ age class drum. There are a few scattered infections in larger and older fishes. This again confirms our earlier reports that "young of the year" sheepshead are most heavily infected with adult worms having fed on infected copepods the previous fall. This inference also fits with a study of the size ranges of freshwater-drum feeding on copepods conducted by Price, 1963 (J. W. Price 1963. A study of food habits of some Lake Erie fishes. Bull. Ohio Bio. Sur., New Series. Vol II, No. i-see Table 24, p. 46). The slight incidence of worms in larger and older fish may be due to accidental ingestion of copepods with other foods.

Graph 8 clearly shows that there is a period at the end of August and beginning of September when there are no worms in the eyes of any fish of any size. This marks the end of the generation of *Philometra* for the year. The adult females have streamed from the eyes and released their larvae. The portion of the female which remains in the eye begins to be encapsulated with connective tissue.

Discussion, Frequency Distribution of Juvenile Worms: Many 0+ class freshwater-drum which had developed during the summer of 1972 were infected by ingesting copepods late in July and in August. After penetrating the intestinal wall the worms traverse the body cavity and migrate to the eyes. Graphs 5 and 8 demonstrate that the juvenile stages occurred in the eyes by 4 September, 1972, and that they had a much greater frequency and intensity of infection than the adult worms. Graphs 2 and 7 show that the vast majority of juveniles occur in the eyes of freshwater drum, which range in length from 3 to 10 cm, 0+ age class fish. When the data from these graphs is correlated with Graph 9 it can easily be seen that these are small "young of the year" which developed during the summer.

A small number of worms (11) occurred in fish 14 to 18 cm in length. These fish which appear just above the break in Graph 9 for fish collected in September and October, were probably 1+ age class and possibly 2+ age class. It is interesting to note that the same break in size of infected fishes occurs in Graph 2 as occurred on Graph 9 which shows the sizes of all fishes collected.

Discussion, Encapsulations: Encapsulations adult female worms occur in all sizes and age classes of fish except for samll 0+ age class freshwater-drum. Small drum from 3 to 10 cm had no encapsulations as can be seen on Graphs 3 and 7. This is easily explained for as we have shown above these are the sheepshead which are being infected for the first time. There is an increase in intensity and incidence of encapsulations in the size range 10 to 14 cm, this is the size range where the worms first become adult and gravid and hang out the eyes. Encapsulations occur in all sizes and age classes of fish examined.

Graphs 6 and 8 illustrate that encapsulations occur in the eyes of fish at all times of the year. This can be explained as reported earlier, the encpasulations occur only in infected fish and are composed of tough connective tissue which may persist in the eyes for years. In older fish these encapsulations denote an earlier infection.

The compterized OMNITAB program aided us in computing the total numbers and percentages of freshwater-drum naturally infected with three categories of *Philometra* sp. Larval or juvenile worms; mature adult female worms and encapsulations of previous infections. The data utilized was accumulated from visual autopsies of fish during the summer of 1972, 1 June through 24 October 1972. The data is presented in tabular form and correlates with Graphs 1 through 9. Where further explanation is deemed necessary a short disqussion follows the appropriate tables.

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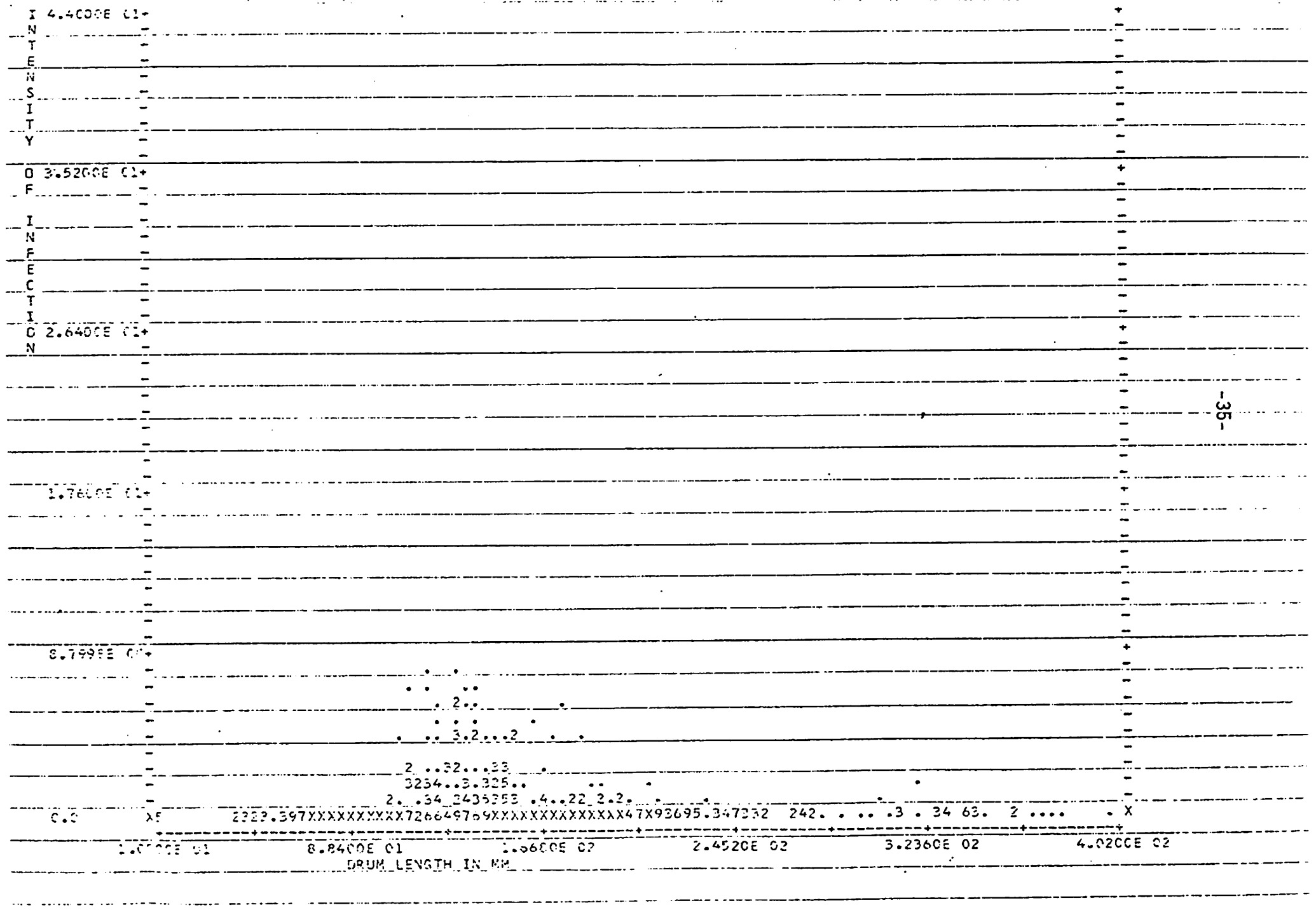

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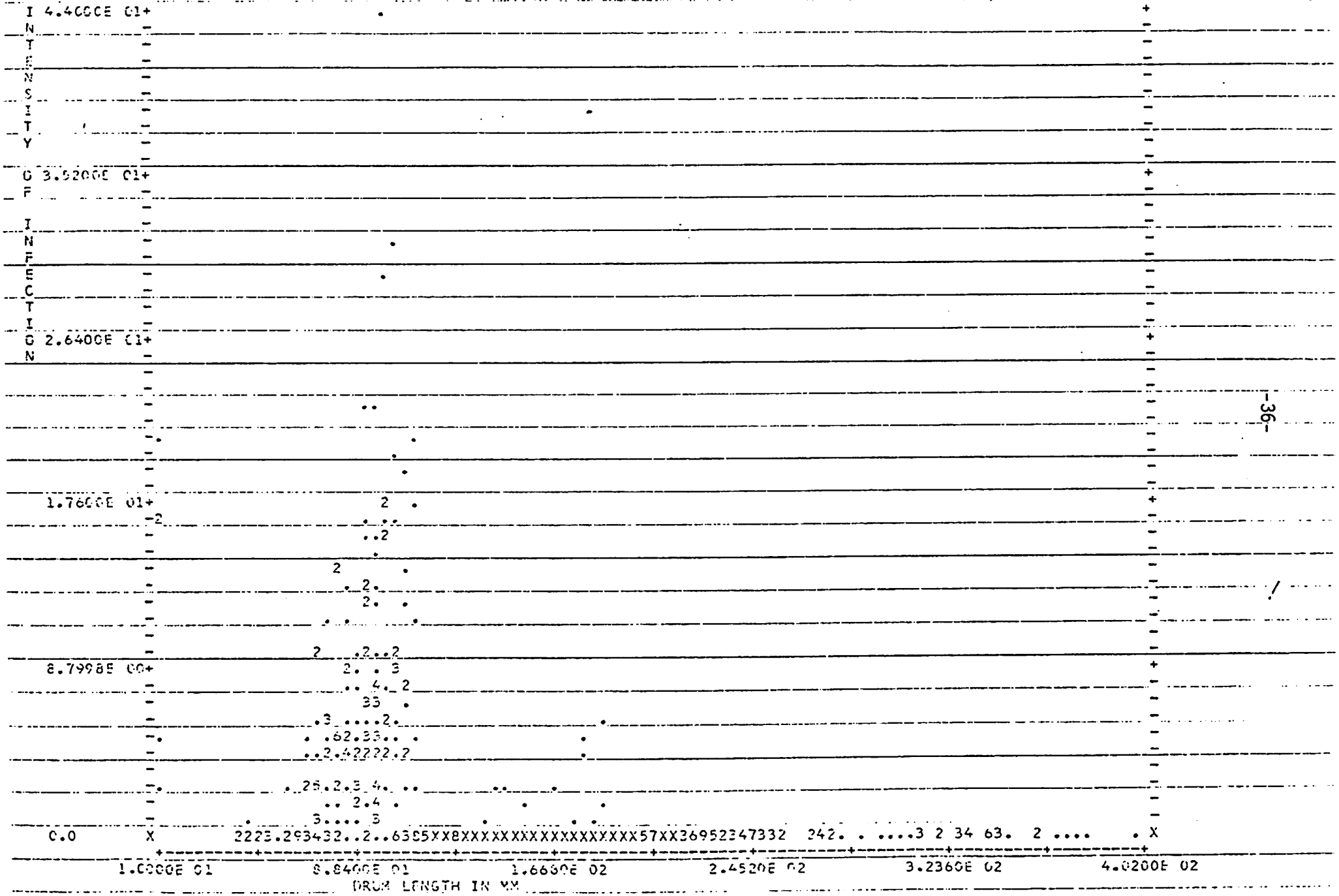

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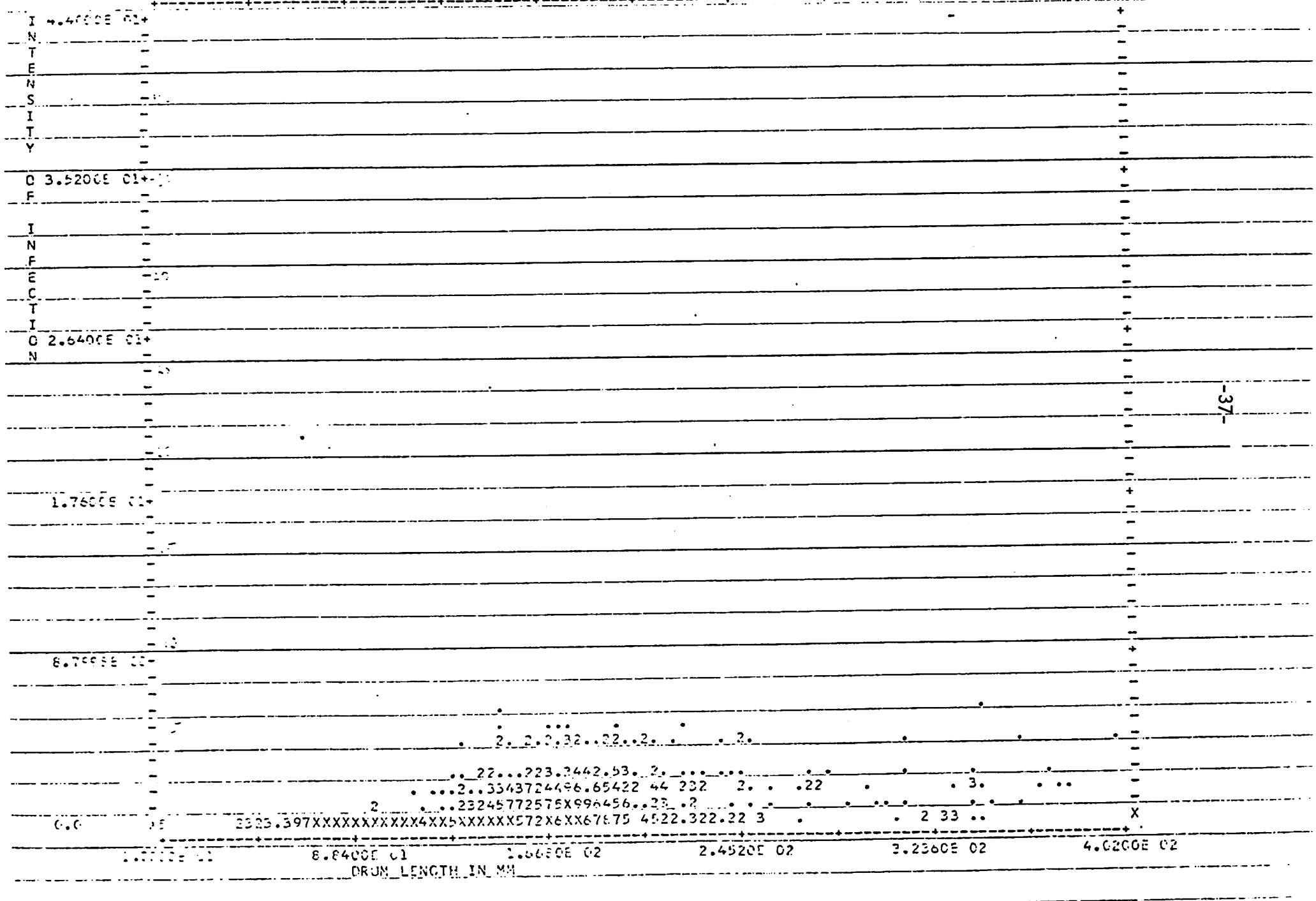

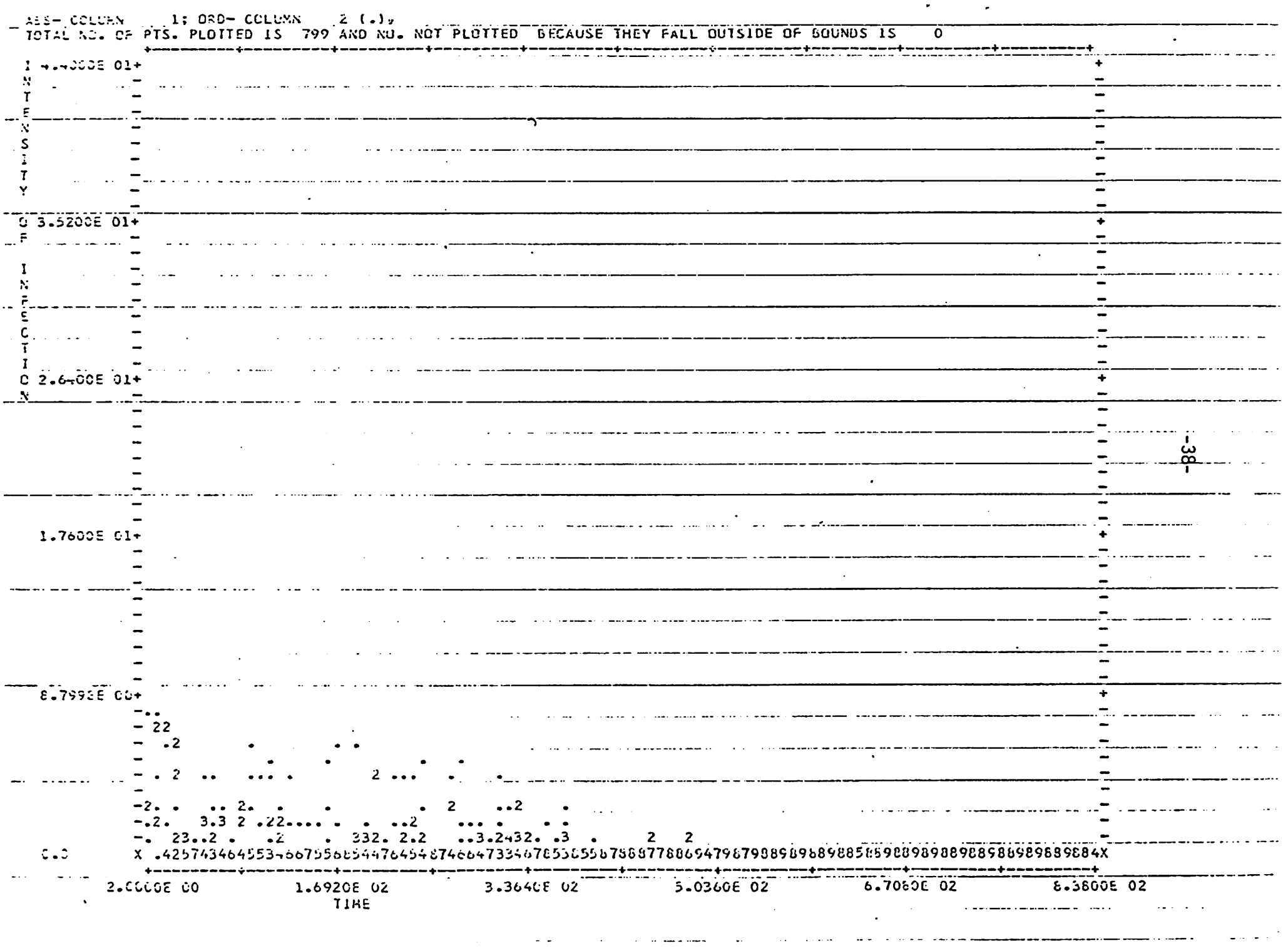
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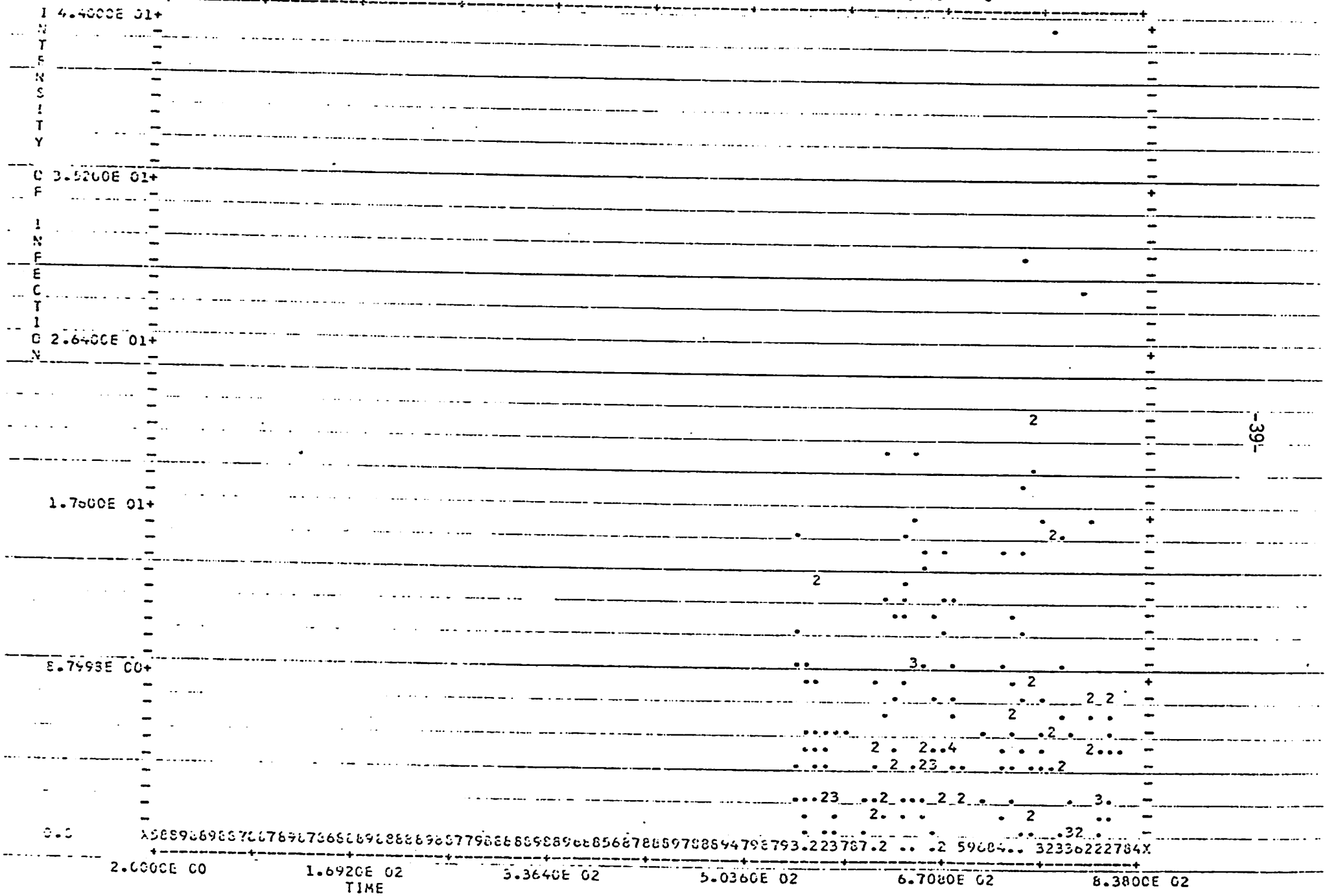
INTENSITY OF INFECTION
3.5200E 01
2.6400E 01
1.7600E 01
8.7998E 00
0.0
2.0000E 00
1.6920E 02
3.3840E 02
5.0760E 02
6.7680E 02
8.3600E 02
TIME

ABS= COLUMN 1; ORD= COLUMN 3 (.),
TOTAL NO. OF PTS. PLOTTED IS 799 AND NO. NOT PLOTTED BECAUSE THEY FALL OUTSIDE OF BOUNDS IS 0



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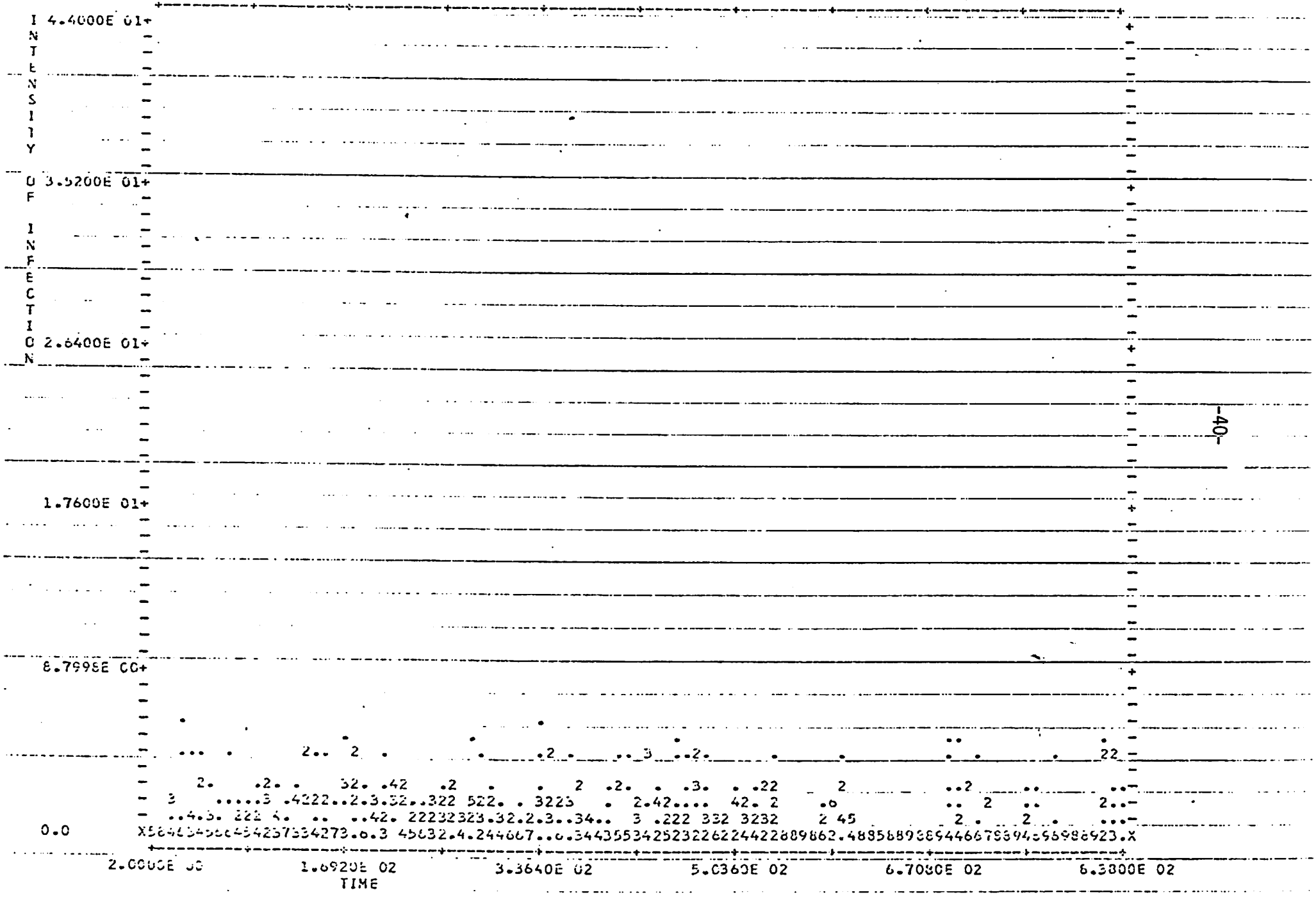
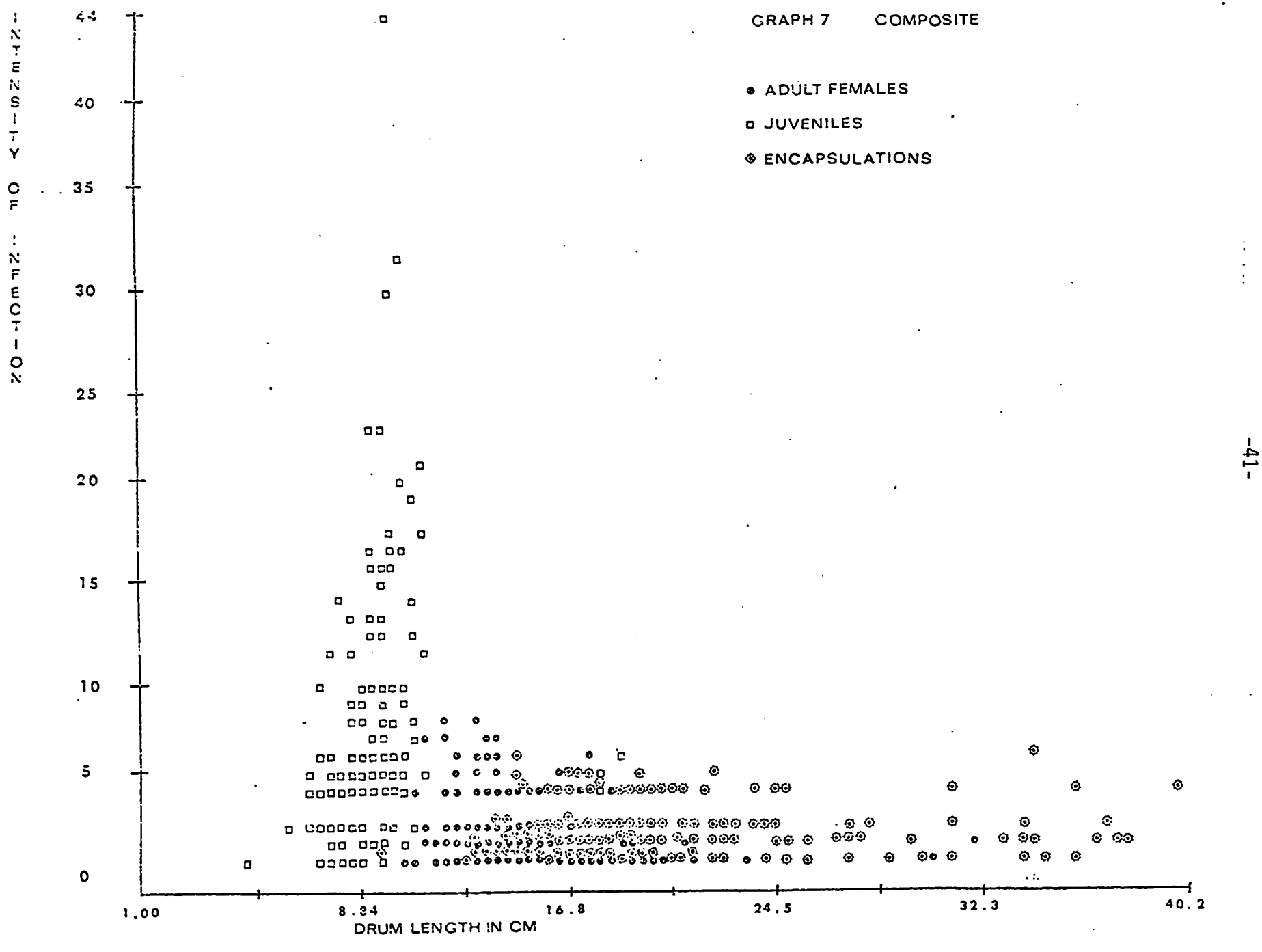
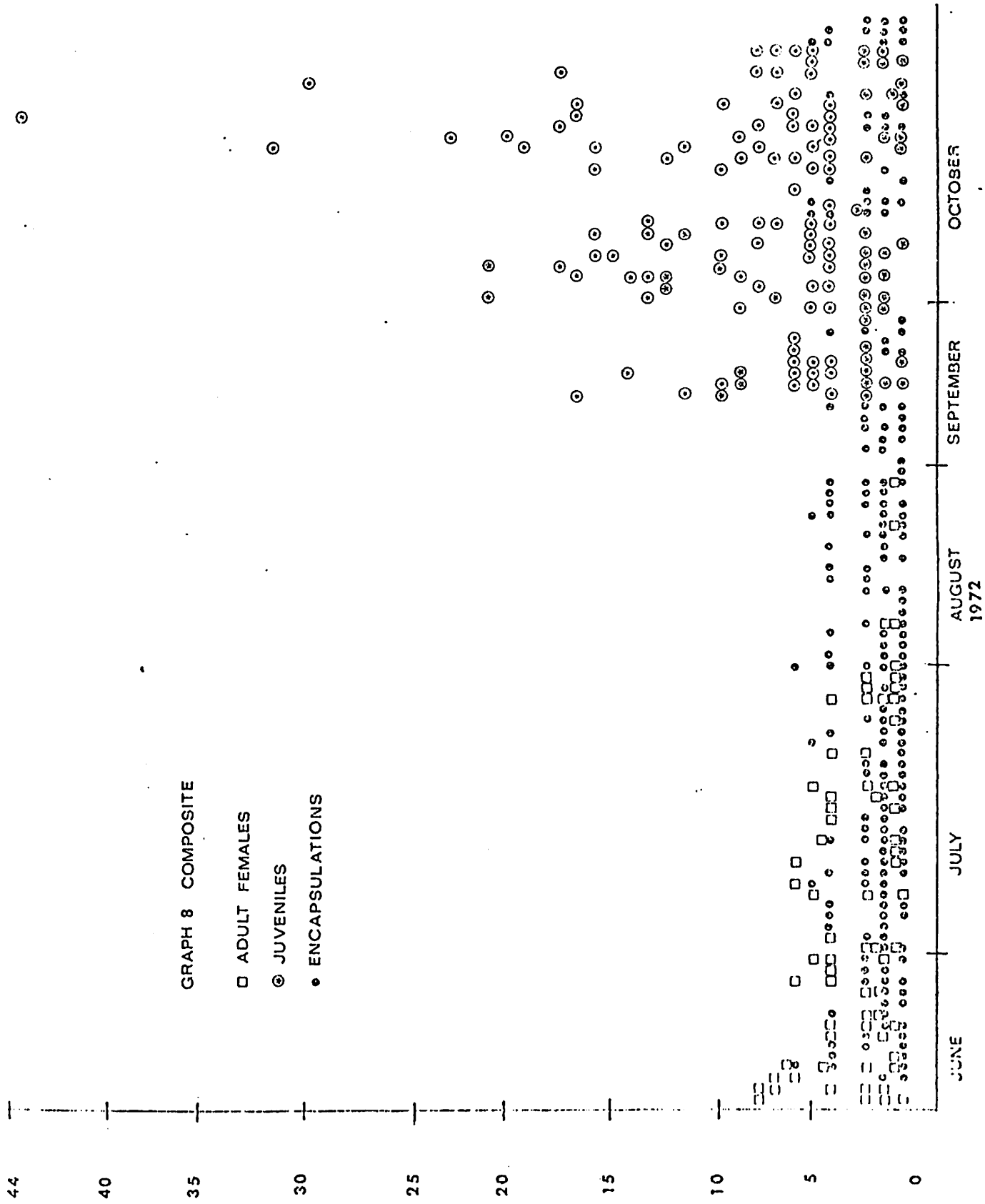



GRAPH 7 COMPOSITE
ADULT FEMALES
JUVENILES
ENCAPSULATIONS
NUMBER OF ANIMALS
0
5
10
15
20
25
30
35
40
44
1.00
8.24
16.8
24.5
32.3
40.2
DRUM LENGTH IN CM


GRAPH 8 COMPOSITE
□ ADULT FEMALES
◎ JUVENILES
• ENCAPSULATIONS
INTENSITY OF INFECTION
44
40
35
30
25
20
15
10
5
0
JUNE
JULY
AUGUST
1972
SEPTEMBER
OCTOBER

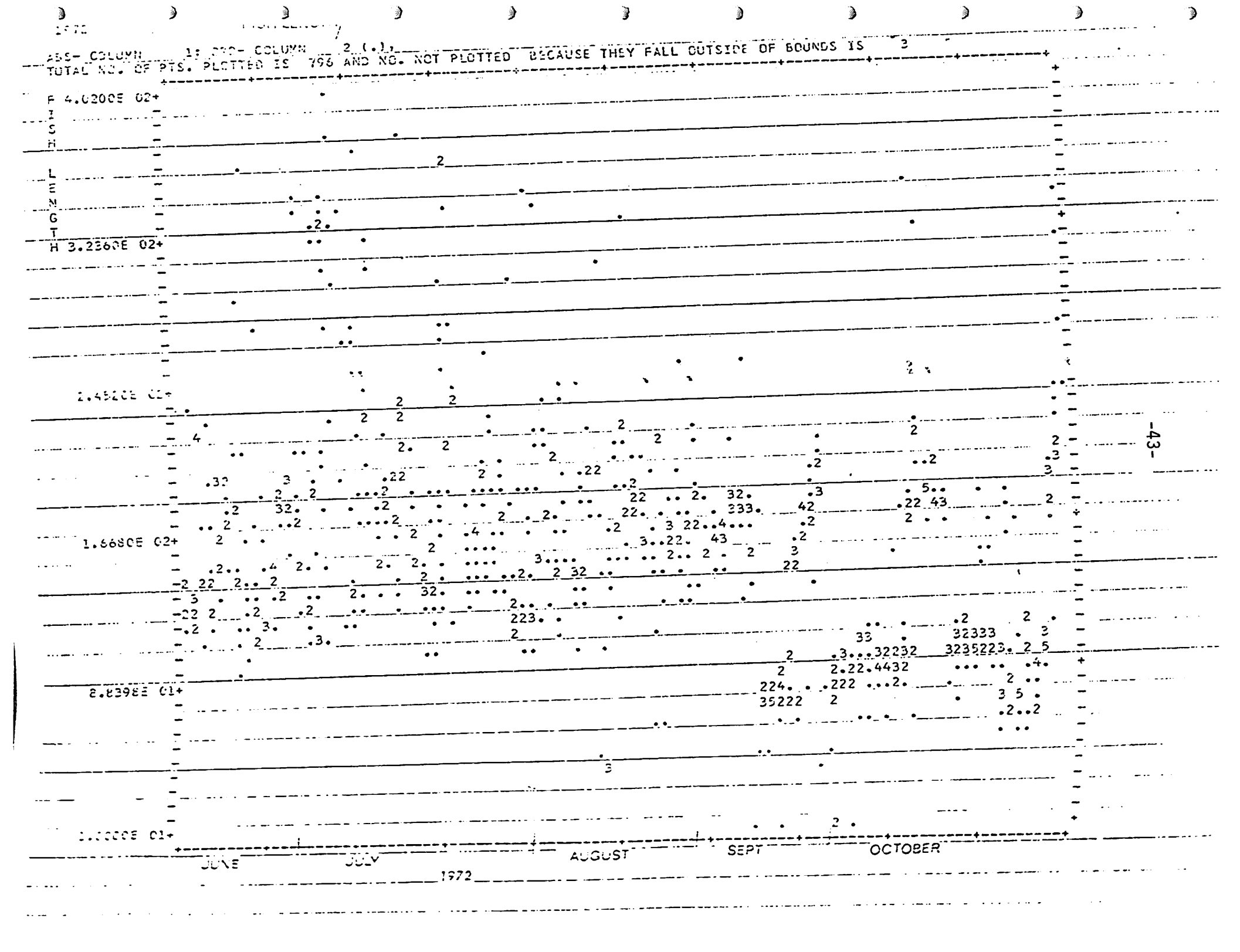

1972
ABS- COLUMN 1; ORD- COLUMN 2 (.).
TOTAL NO. OF PTS. PLOTTED IS 796 AND NO. NOT PLOTTED BECAUSE THEY FALL OUTSIDE OF BOUNDS IS 3
F 4.0200E 02+
I
S
H
L
E
N
G
T
H 3.2360E 02+
2.4520E 02+
1.6680E 02+
8.8398E 01+
1.0000E 01+
JUNE
JULY
AUGUST
SEPT
OCTOBER
1972

TABLE 1
TOTAL NUMBER OF ALL *APLODINOTUS GRUNNIENS* EXAMINED FROM JUNE THROUGH OCTOBER, 1972

1.	Total number of freshwater-drum visually autopsied	799
2.	Total number of freshwater-drum experimentally infected	39
3.	Total number of freshwater-drum examined by digestion technique	30
	Total	868

.....

TABLE 2
TOTAL NUMBER OF INFECTED *APLODINOTUS GRUNNIENS* FROM JUNE THROUGH OCTOBER, 1972

1.	Total number of fish infected with juveniles	175
2.	Total number of fish infected with mature females	141
3.	Total number of fish infected with encapsulations	334
4.	Total number of fish infected with juveniles and adults (mature females)	317
5.	Total number of fish infected with *Philometra* sp., all three stages (This includes some fish with both living mature females and encapsulations in the same eye)	600

Discussion: Items 1, 2, and 3 in Table 2 will correlate with the frequency distributions, Graphs 1 through 9. Item 5 above, as noted, includes some fish which were infected with both mature female worms and encapsulations at the same time. Approximately 6 percent of the total of 600 given above fall into this category. Later in our overall percentage data these two catagories are separated out.

.....

TABLE 3
TOTAL NUMBER OF INFECTED *APLODINOTUS GRUNNIENS*, CATAGORIZED BY EYE INFECTED

1.	Total number of fish infected with mature females (Adults) in the right eye only.	46
2.	Total number of fish infected with mature females (Adults) in the left eye only.	66
3.	Total number of fish infected with juveniles in the right eye only.	14
4.	Total number of fish infected with juveniles in the left eye only.	14

TABLE 3
Continued

5.	Total number of fish infected with encapsulations in the right eye only.	69
6.	Total number of fish infected with encapsulations in the left eye only.	98
7.	Total number of fish infected with mature females (Adults) in the right eye (double and single).	95
8.	Total number of fish infected with mature females (Adults) in the left eye only (double and single).	106
9.	Total number of fish infected with juveniles in the right eye (double and single).	162
10.	Total number of fish infected with juveniles in the left eye (double and single).	162
11.	Total number of fish infected with encapsulations in the right eye (double and single).	235
12.	Total number of fish infected with encapsulations in the left eye (double and single).	264
13.	Total number of fish infected with mature females (Adults) in both eyes (double).	60
14.	Total number of fish infected with juveniles in both eyes (double).	148
15.	Total number of fish infected with encapsulations in both eyes (double).	

.....

TABLE 4
TOTAL NUMBER OF *PHILOMETRA* STAGES PRESENT IN ALL *APLODINOTUS GRUNNIENS* AUTOPSIED FROM JUNE THROUGH OCTOBER, 1972

1.	Total number of adults in the right eye only.	46
2.	Total number of adults in the left eye only.	66
3.	Total number of juveniles in the right eye only.	37
4.	Total number of juveniles in the left eye only.	31
5.	Total number of encapsulations in the right eye only.	86
6.	Total number of encapsulations in the left eye only.	129
7.	Total number of adults in double eye infections.	235
8.	Total number of juveniles in double eye infections.	1218
9.	Total number of encapsulations in double eye infections.	479
10.	Total number of adults in the right eye (double and single eye infections).	167
11.	Total number of adults in the left eye double and single eye infections).	180

TABLE 4
Continued

12. Total number of juveniles in the right eye (double and single eye infections).	591
13. Total number of juveniles in the left eye (double and single infections).	695
14. Total number of encapsulations in the right eye (double and single eye infections).	330
15. Total number of encapsulations in the left eye (double and single eye infections).	364

.....

TABLE 5
SUMMARY OF SOME IMPORTANT TOTALS

	DOUBLE EYE INFECTIONS		INFECTIONS RIGHT EYE ONLY		INFECTIONS LEFT EYE ONLY	
	Worms	Fish	Worms	Fish	Worms	Fish
Adults	235	60	46	35	66	46
Juveniles	1218	148	37	14	31	14
Encapsulations	479	166	86	69	129	98

.....

TABLE 6
INTENSITY OF INFECTIONS WITH MATURE FEMALES (ADULTS)
DURING THE PERIOD JUNE THROUGH JULY, 1972

Total number of worms in the right eye only.	46
Total number of fish with worms in the right eye only.	35
Average intensity of infection in the right eye only.	1.31*

.....

Total number of worms in the left eye only.	66
Total number of fish with worms in the left eye only.	46
Average intensity of infection in the left eye only.	1.43*

.....

Total number of worms in double eye infections only.	235
Total number of fish with double eye infections only.	60
Average intensity of infection with adult worms in double eye infections only.	3.91

.....

*Tested using Chi-Square there is no significant difference at the 5% level.

TABLE 6
Continued

Total number of right eye infections (single and double).	167
Total number of fish with right eye infections (single and double).	95
Average intensity of infection in the right eye (single and double).	1.75*

.....

Total number of left eye infections (single and double).	180
Total number of fish with left eye infections (single and double).	106
Average intensity of infection in the left eye (single and double).	1.69*

.....

Discussion: The data presented here (Table 6) demonstrates that there is no preference for eith right or left eyes. It also indicates that the average intensity of infection is higher when both eyes are infected.

.....

*Tested using Chi-Square there is no significant difference at the 5% level.

TABLE 7
INTENSITY OF INFECTIONS WITH"JUVENILE" WORMS
DURING THE PERIOD AUGUST THROUGH SEPTEMBER, 1972

Total number of worms in the right eye only.	37
Total number of fish with worms in the right eye only.	14
Average intensity of infection with juveniles in the right eye only.	2.64*

.....

Total number of worms in left eye infections only.	31
Total number of fish with worms in the left eye only.	14
Average intensity of infection with juveniles in the left eye only.	2.21*

.....

*Tested using Chi-Square there is no significant difference at the 5% level.

TABLE 7
Continued

Total number of worms in double eye infections only	1218
Total number of fish with double eye infections only	148
Average intensity of infection with juvenile worms in double infections only.	8.20
.....	
Total number of right eye infections (single and double).	591
Total number of fish with right eye infections (single and double).	
Average intensity of infection in the right eye (single and double).	3.03
.....	
Total number of left eye infections (single and double).	695
Total number of fish with left eye infections (single and double).	162
Average intensity of infection in the left eye (single and double).	4.28
.....	

Discussion: The data presented here (Table 7) illustrates that there is no strong preference by juvenile philometrids for either right or left eyes. It also indicates that the average intensity of infection and incidence of infection is higher for double eye infections. When average intensity of infection for juveniles, 8.20 is compared with average intensity of infection for adults, 3.91 (Table 6) it is apparent that the intensity is higher for juvenile worms.

.....

TABLE 8
INTENSITY OF INFECTIONS WITH ENCAPSULATIONS OF SPENT FEMALE WORMS, JUNE THROUGH OCTOBER, 1972

Total number of encapsulations in the right eye only.	86
Total number of fish with encapsulations in the right eye only.	69
Average intensity of infection with encapsulations in the right eye only.	1.25*

*Tested using Chi-Square there is no significant difference at the 5% level.

TABLE 8
Continued

Total number of encapsulations in the left eye only.	129
Total number of fish with encapsulations in the left eye only.	98
Average intensity of infection with encapsulations in the left eye only.	1.32*
.....	
Total number of encapsulations in double eye infections only.	479
Total number of fish with encapsulations in double eye infections only.	166
Average intensity of infection with encapsulations in double eye infections only.	2.88
.....	
Total number of encapsulations in right eye infections (single and double).	330
Total number of fish with right eye infections with encapsulations (single and double).	235
Average intensity of infection encapsulations in the right eye (single and double).	1.40*
.....	
Total number of encapsulations in left eye infections (single and double).	364
Total number of fish with encapsulations in left eye (single and double).	264
Average intensity of infection with encapsulations in the left eye (single and double).	1.38*
.....	

*Tested using Chi-Square there is no significant difference at the 5% level.

Discussion: The data presented here correlates with that given in Table 6, there is no significant preference for either right or left eyes for encapsulation. This is to be expected as the mature gravid females which partially stream from the eyes show no preference. As in the case of the mature females there is a slightly higher intensity and incidence of infection for double eye infections.

TABLE 9
INCIDENCE OF INFECTION (PERCENTAGE) OF ALL STAGES OF *PHILOMETRA* SP. IN *APLODINOTUS GRUNNIENS*, JUNE THROUGH OCTOBER, 1972

1.	Percent of all fish infected with juveniles.	22%
2.	Percent of all fish infected with adults.	17.5%
3.	Percent of all fish infected with encapsulations.	42%
4.	Percent of all fish infected with juveniles and adults.	39.7%
5.	Percent of all fish infected with *Philometra* (all three stages). Adults and encapsulations figured separately.	82%
6.	Percent of all fish infected with *Philometra* (all three stages). Considering cases (6%) where adults and encapsulations occur in the same eye.	76.4%
7.	Percent of all fish with adults in the right eye only.	4.37%
8.	Percent of all fish infected with adults in the left eye only.	5.7%
9.	Percent of all fish infected with adults in both eyes only.	7.5%
10.	Percent of all fish with juveniles in the right eye only.	1.7%
11.	Percent of all fish with juveniles in the left eye only.	1.7%
12.	Percent of all fish with juveniles in both eyes.	18.5%
13.	Percent of all fish with encapsulations in the right eye only.	8.6%
14.	Percent of all fish with encapsulations in the left eye only.	12.2%
15.	Percent of all fish with encapsulations in both eyes.	20.8%
16.	Percent of fish infected with adult females that have right eye infections only.	24.8%
17.	Percent of fish infected with adult females that have left eye infections only.	32.5%
18.	Percent of fish infected with adult females that have double eye infections.	42.5%
19.	Percent of fish infected with juveniles that have infections in the right eye only.	8.0%
20.	Percent of all fish infected with juveniles that have infections in the left eye only.	8.0%
21.	Percent of fish infected with juveniles that have double eye infections.	84.5%

TABLE 9
Continued

22.	Percent of fish infected with encapsulations that have right eye infections only.	20.6%
23.	Percent on fish infected with encapsulations that have left eye infections only.	29.3%
24.	Percent of fish infected with encapsulations that have double eye infections.	50.2%

Discussion: These percentage of incidence figures correlate quite well with the frequency distribution, Graphs 1-9. The apparent disagreement be tween items 2 and 3, Table 9, can easily be explained if one considers the adult females were present in only June and July of 1972, while encapsulations were present in several age classes of fish throughout the entire sampling period.

While the data presented in Tables 6, 7, and 8 show no preference for right or left eyes for intensity of infection the situation may be different for incidence of infection. In this table (Table 9) items 10, 11, 12, and 19, 20, 21 indicate there is no difference in incidence for the occurrence of juveniles. However, all other items, in the series 7 through 24 indicate that there may be a preference for incidence in left eye over right eye, more fish were infected with adult females and encapsulations in the left eye.

One of the questions most often asked concerning this Study I of Project F-48-R is whether or not there is a preference by *Philometra* sp. for either right or left eyes or whether either is more common than double eye infections. Tables 6, 7 and 8 present the data for the infections and Graphs 10 and 11 present it in graphic form.

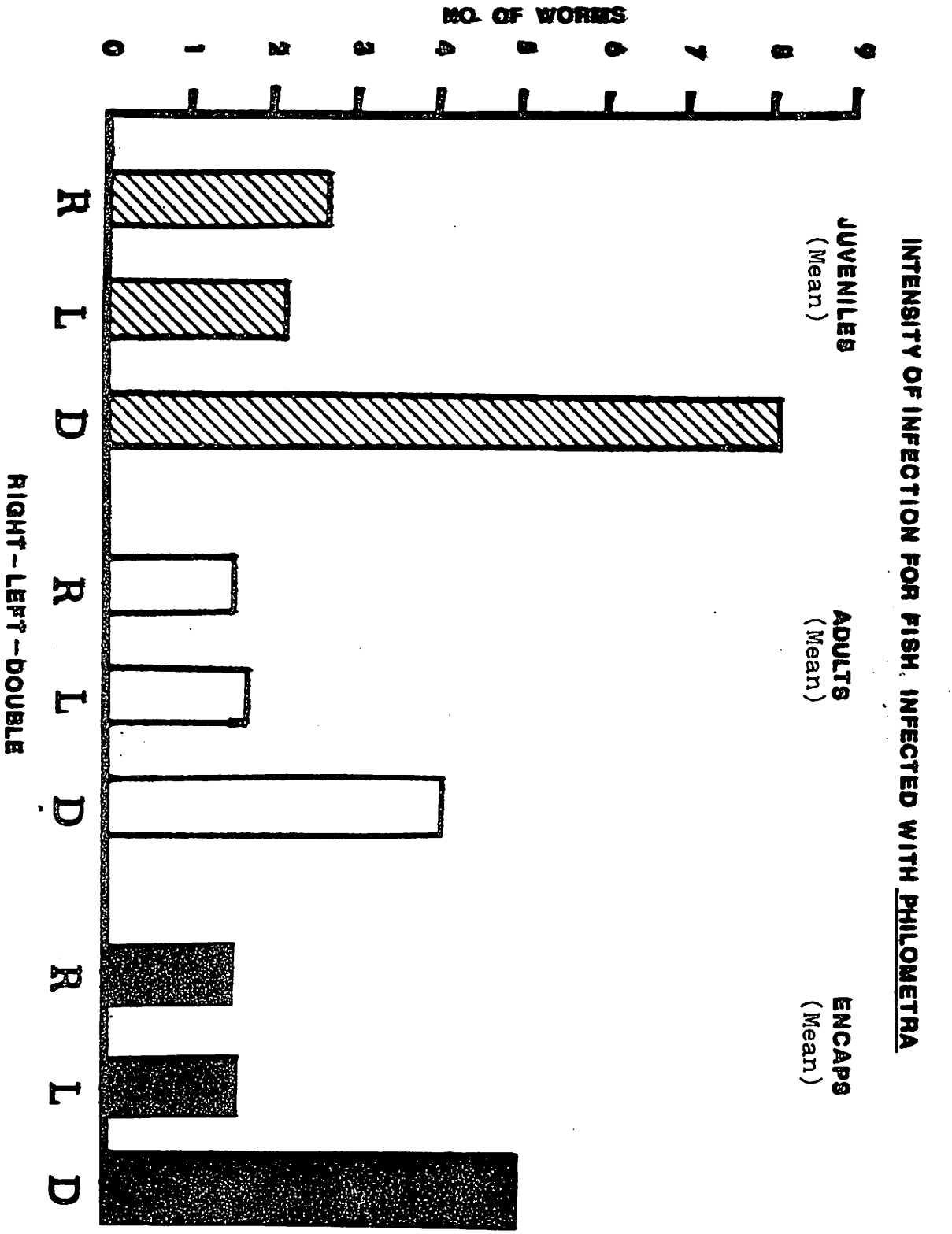
Graph 10 shows the mean intensity of infection for right eye only, left eye only and double eye only infections for juveniles, adults and encapsulations of *Philometra* sp. The data is from all infected fish of all sized autopsied during the period June through October 1972. There is no significant difference (tested with chi-square) between right and left eye infections for juveniles, adults and encapsulations. In each life history phase there is a significant difference when the mean intensities of right and left eyes are compared with double eye infections. Mean intensity of infection was always higher in double eye infections. If one considers the intensity of infection in juvenile worms alone the mean intensity of infection in double eye infections is more than 3 times that found in either the right or left eyes. The mean worm burden when both eyes are infected is more than just a sum of the mean burden in the two eyes only. At present we have no biological or ecological explanation for this. When the average intensity for juveniles, 8.2 is compared with the average intensity of infection for adults, 3.91, it is apparent that the mean intensity of infection is higher for juveniles. This indicates that there is not only a loss of male nematodes, which are very few in number, but also a loss of females over the winter period. This leads one to suspect that there may be a density dependent factor which comes into force as the worms begin to grow in the early spring. In both our April and May samples this year we did find medium sized and fairly fresh encapsulations in the eyes. These encapsulations may represent females lost over the winter months.

Graph 10 also illustrates that mean intensity of encapsulations in the eyes almost parallels the intensities for adult females. This is to be expected as it is a portion of the spent adult gravid female which remains in the eye which forms the nucleus about which the connective tissue encapsulation forms.

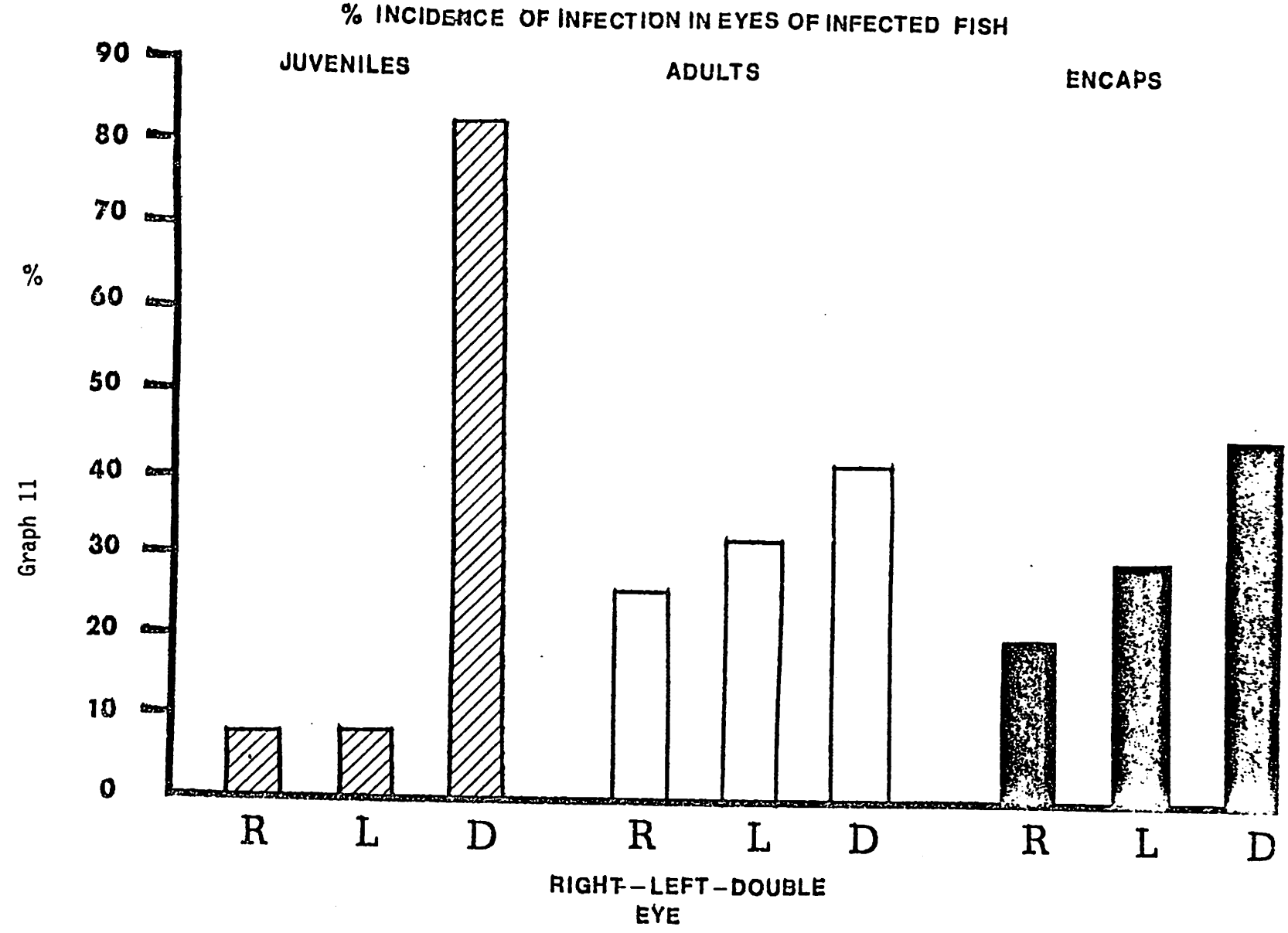
Graph 11 illustrates the incidence of infection, the percentage of infected fish with right, left and double eye infections in the period June through October 1972. The incidence of infection with juveniles in both right and left eyes is 8 percent each, again there is no significant preference for either right or left eyes. Double eye infections occur with an incidence of 84 percent, 84 percent of the fish infected with juvenile philometrids had juvenile worms in both eyes. This is more than 10 times the number of fish with juvenile worms in either the right or left eyes alone. There is clearly a preference for double eye infections as indicated by mean intensity of infection and by incidence of infection.

When one considers the incidence of adult worms and encapsulations the situation is somewhat different. Again the highest incidence was in double eye infections. In the case of both adults and encapsulations the incidence is lower for double eye infections and higher for single eye infections than it was for juveniles. Again the incidences for encapsulations parallels the incidence for adult females as would be expected. These facts indicate two things which must be investigated in more detail in our work this coming year. It appears, as stated before, that there is some loss of worms over the winter months and that the loss is differential.

Graph 10


INTENSITY OF INFECTION FOR FISH INFECTED WITH PHILOMETRA
NO. OF WORMS
0 1 2 3 4 5 6 7 8 9
JUVENILES (Mean)
ADULTS (Mean)
ENCAPS (Mean)
R L D R L D R L D
RIGHT-LEFT-DOUBLE EYE

Graph 11


% INCIDENCE OF INFECTION IN EYES OF INFECTED FISH
JUVENILES
ADULTS
ENCAPS
%
90
80
70
60
50
40
30
20
10
0
R
L
D
R
L
D
R
L
D
RIGHT—LEFT—DOUBLE EYE

The loss occurs more often in double eye infections than in single eye infections which lowers the incidence of double eye infections by the spring and early summer months. This seems to happen in one eye of a double eye infection and accounts for the higher percentage of single eye infections with adults compared with juveniles. There also seems to be a higher incidence in the left eye than in the right eye. This should be checked again this next year to see if it was chance alone this year or if this is an actual incidence preference of adult philometrids for left eyes.

The remainder of this report will be devoted to an analysis of data on a monthly basis. The occurrence of *Philometra* sp. parasitic stages in different fish host size-classes are given for each month during the collecting period.

A computer program was developed using FORTRAN language to compile this data in usuable form. Tables 10 for juvenile stages, 11 for adult females and 12 for encapsulations are the result. On each Table there is a box or group of figures for each size-class for each month of the year. Each box except the last one for each month is composed of five lines, each line is self explanatory with perhaps the exception of the line reading "fraction", the fraction is the percentage of infection for that class in that month. Smaller and more concise tables were developed from each of the larger ones giving the essential data for purposes of discussion.

Tables 13, 14, and 15 were derived from Table 10.

TABLE 10

FISH INFECTED WITH JUVENILE WORMS

CLASS	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER
-5 CM	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.251 MEAN WORMS= -0.0 VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.251 MEAN WORMS= -0.0 VARIANCE= 0.000 STD.DEV.=******	NO. OF FISH= 4.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 6.0 NO. INFECTED= 5.0 FRACTION=0.833 MEAN WORMS= 7.8 VARIANCE=66.139 STD.DEV.= 8.133	NO. OF FISH= 1.0 NO. INFECTED= 1. FRACTION=1.000 MEAN WORMS= 17.0 VARIANCE= 0.000 STD.DEV.= 0.016
1--10	NO. OF FISH= 1.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.886 MEAN WORMS= -0.0 VARIANCE=****** STD.DEV.=-0.000	NO. OF FISH= 2.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 50.0 NO. INFECTED= 42.0 FRACTION=0.840 MEAN WORMS= 4.5 VARIANCE=13.690 STD.DEV.= 3.700	NO. OF FISH=105.0 NO. INFECTED= 83. FRACTION=0.790 MEAN WORMS= 5.4 VARIANCE=28.697 STD.DEV.= 5.357
1--15	NO. OF FISH= 51.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 77.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 38.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 13.0 NO. INFECTED= 3.0 FRACTION=0.231 MEAN WORMS= 0.5 VARIANCE= 1.172 STD.DEV.= 1.082	NO. OF FISH= 35.0 NO. INFECTED= 34. FRACTION=0.971 MEAN WORMS= 11.3 VARIANCE=85.540 STD.DEV.= 9.249
1--20	NO. OF FISH= 42.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 74.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 69.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 55.0 NO. INFECTED= 1.0 FRACTION=0.018 MEAN WORMS= 0.0 VARIANCE= 0.071 STD.DEV.= 0.267	NO. OF FISH= 47.0 NO. INFECTED= 7.0 FRACTION=0.149 MEAN WORMS= 0.5 VARIANCE= 1.738 STD.DEV.= 1.318
1--25	NO. OF FISH= 9.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 30.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 24.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 3.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 12.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0
-30	NO. OF FISH= 2.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 12.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 1.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 1.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 1.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0
-35	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS= 2.0 VARIANCE= 0.000 STD.DEV.=******	NO. OF FISH= 17.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 3.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS=***** VARIANCE=****** STD.DEV.=******	NO. OF FISH= 4.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0
-40	NO. OF FISH= 1.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 6.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS=***** VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS=***** VARIANCE=****** STD.DEV.=-0.000	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.000 MEAN WORMS= 0.0 VARIANCE= 0.000 STD.DEV.=-0.000
-45	NO. OF FISH= 1.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS= 0.1 VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS= 1.1 VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=-.000 MEAN WORMS= -0.0 VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS=***** VARIANCE= 0.000 STD.DEV.=******
	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.251 MEAN WORMS= -0.0 VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS= 64.2 VARIANCE=****** STD.DEV.= 0.000	NO. OF FISH= 2.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=-.000 MEAN WORMS= -0.0 VARIANCE=-0.000 STD.DEV.=-7.907	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.000 MEAN WORMS= -0.0 VARIANCE= 0.000 STD.DEV.=******
	TOTAL FISH=107.0 INFECTED FISH= 0.0 FRACTION=0.0	TOTAL FISH=216.0 INFECTED FISH= 0.0 FRACTION=0.0	TOTAL FISH=143.0 INFECTED FISH= 0.0 FRACTION=0.0	TOTAL FISH=128.0 INFECTED FISH= 51.0 FRACTION=0.398	TOTAL FISH=205.0 INFECTED FISH=125.0 FRACTION=0.610

TABLE 11

FISH INFECTED WITH ADULT WORMS

SIZE CLASS	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER
0-5 CM	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.251 MEAN WORMS= -0.0 VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.251 MEAN WORMS= -0.0 VARIANCE= 0.000 STD.DEV.=******	NO. OF FISH= 4.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 6.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= NO. INFECTED= FRACTION=0.0 MEAN WORMS= 0. VARIANCE= 0.0 STD.DEV.= 0.0
5.1-10	NO. OF FISH= 1.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.886 MEAN WORMS= -0.0 VARIANCE=****** STD.DEV.=-0.000	NO. OF FISH= 2.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 50.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH=105 NO. INFECTED= FRACTION=0.0 MEAN WORMS= 0. VARIANCE= 0.0 STD.DEV.= 0.0
10.1-15	NO. OF FISH= 51.0 NO. INFECTED= 39.0 FRACTION=0.765 MEAN WORMS= 2.6 VARIANCE= 5.567 STD.DEV.= 2.359	NO. OF FISH= 77.0 NO. INFECTED= 49.0 FRACTION=0.636 MEAN WORMS= 1.5 VARIANCE= 2.715 STD.DEV.= 1.648	NO. OF FISH= 38.0 NO. INFECTED= 10.0 FRACTION=0.263 MEAN WORMS= 0.4 VARIANCE= 0.496 STD.DEV.= 0.704	NO. OF FISH= 13.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 35. NO. INFECTED= 0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0
15.1-20	NO. OF FISH= 42.0 NO. INFECTED= 15.0 FRACTION=0.357 MEAN WORMS= 0.8 VARIANCE= 2.091 STD.DEV.= 1.446	NO. OF FISH= 74.0 NO. INFECTED= 21.0 FRACTION=0.284 MEAN WORMS= 0.5 VARIANCE= 1.007 STD.DEV.= 1.003	NO. OF FISH= 69.0 NO. INFECTED= 1.0 FRACTION=0.014 MEAN WORMS= 0.0 VARIANCE= 0.014 STD.DEV.= 0.120	NO. OF FISH= 55.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 47. NO. INFECTED= 0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0
20.1-25	NO. OF FISH= 9.0 NO. INFECTED= 1.0 FRACTION=0.111 MEAN WORMS= 0.2 VARIANCE= 0.395 STD.DEV.= 0.629	NO. OF FISH= 30.0 NO. INFECTED= 2.0 FRACTION=0.067 MEAN WORMS= 0.1 VARIANCE= 0.062 STD.DEV.= 0.249	NO. OF FISH= 24.0 NO. INFECTED= 1.0 FRACTION=0.042 MEAN WORMS= 0.0 VARIANCE= 0.040 STD.DEV.= 0.200	NO. OF FISH= 3.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 12.0 NO. INFECTED= 0. FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0
25.1-30	NO. OF FISH= 2.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 12.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 1.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 1.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 1.0 NO. INFECTED= 0. FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0
30.1-35	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS= 2.0 VARIANCE= 0.000 STD.DEV.=******	NO. OF FISH= 17.0 NO. INFECTED= 2.0 FRACTION=0.118 MEAN WORMS= 0.2 VARIANCE= 0.263 STD.DEV.= 0.513	NO. OF FISH= 3.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS=***** VARIANCE=****** STD.DEV.=******	NO. OF FISH= 4.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0
35.1-40	NO. OF FISH= 1.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 6.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS=***** VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS=***** VARIANCE=****** STD.DEV.=-0.000	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.000 MEAN WORMS= 0.0 VARIANCE= 0.000 STD.DEV.=-0.000
40.1-45	NO. OF FISH= 1.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS= 0.1 VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS= 1.1 VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=-.000 MEAN WORMS= -0.0 VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=****** MEAN WORMS=***** VARIANCE= 0.000 STD.DEV.=******
45.1-	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.251 MEAN WORMS= -0.0 VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS= 64.2 VARIANCE=****** STD.DEV.= 0.000	NO. OF FISH= 2.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=-.000 MEAN WORMS= -0.0 VARIANCE=-0.000 STD.DEV.=-7.907	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.000 MEAN WORMS= -0.0 VARIANCE= 0.000 STD.DEV.=******
	TOTAL FISH=107.0 INFECTED FISH= 55.0 FRACTION=0.514	TOTAL FISH=216.0 INFECTED FISH= 74.0 FRACTION=0.343	TOTAL FISH=143.0 INFECTED FISH= 12.0 FRACTION=0.084	TOTAL FISH=128.0 INFECTED FISH= 0.0 FRACTION=0.0	TOTAL FISH=205.0 INFECTED FISH= 0.0 FRACTION=0.0

FISH INFECTED WITH ENCAPSULATED WORMS PAGE 3

TABLE 12

IZE CLASS	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER
)-5 CM	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.251 MEAN WORMS= -0.0 VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.251 MEAN WORMS= -0.0 VARIANCE= 0.000 STD.DEV.=******	NO. OF FISH= 4.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 6.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 1.0 NO. INFECTED= 0. FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0
.1-10	NO. OF FISH= 1.0 NO. INFECTED= 1.0 FRACTION=1.000 MEAN WORMS= 1.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.886 MEAN WORMS= -0.0 VARIANCE=****** STD.DEV.=-0.000	NO. OF FISH= 2.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 50.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH=105.0 NO. INFECTED= 1. FRACTION=0.010 MEAN WORMS= 0.0 VARIANCE= 0.009 STD.DEV.= 0.097
.1-15	NO. OF FISH= 51.0 NO. INFECTED= 6.0 FRACTION=0.118 MEAN WORMS= 0.3 VARIANCE= 1.070 STD.DEV.= 1.035	NO. OF FISH= 77.0 NO. INFECTED= 17.0 FRACTION=0.221 MEAN WORMS= 0.3 VARIANCE= 0.457 STD.DEV.= 0.676	NO. OF FISH= 38.0 NO. INFECTED= 21.0 FRACTION=0.553 MEAN WORMS= 1.3 VARIANCE= 2.036 STD.DEV.= 1.427	NO. OF FISH= 13.0 NO. INFECTED= 7.0 FRACTION=0.538 MEAN WORMS= 1.1 VARIANCE= 1.609 STD.DEV.= 1.269	NO. OF FISH= 35.0 NO. INFECTED= 1. FRACTION=0.029 MEAN WORMS= 0.1 VARIANCE= 0.250 STD.DEV.= 0.500
.1-20	NO. OF FISH= 42.0 NO. INFECTED= 27.0 FRACTION=0.643 MEAN WORMS= 1.1 VARIANCE= 1.248 STD.DEV.= 1.117	NO. OF FISH= 74.0 NO. INFECTED= 51.0 FRACTION=0.689 MEAN WORMS= 1.4 VARIANCE= 1.732 STD.DEV.= 1.316	NO. OF FISH= 69.0 NO. INFECTED= 46.0 FRACTION=0.667 MEAN WORMS= 1.2 VARIANCE= 1.490 STD.DEV.= 1.221	NO. OF FISH= 55.0 NO. INFECTED= 40.0 FRACTION=0.727 MEAN WORMS= 1.3 VARIANCE= 1.159 STD.DEV.= 1.077	NO. OF FISH= 47.0 NO. INFECTED= 27.(FRACTION=0.574 MEAN WORMS= 1.5 VARIANCE= 2.675 STD.DEV.= 1.636
.1-25	NO. OF FISH= 9.0 NO. INFECTED= 7.0 FRACTION=0.778 MEAN WORMS= 1.4 VARIANCE= 0.914 STD.DEV.= 0.956	NO. OF FISH= 30.0 NO. INFECTED= 26.0 FRACTION=0.867 MEAN WORMS= 1.9 VARIANCE= 1.490 STD.DEV.= 1.221	NO. OF FISH= 24.0 NO. INFECTED= 13.0 FRACTION=0.542 MEAN WORMS= 1.5 VARIANCE= 2.582 STD.DEV.= 1.607	NO. OF FISH= 3.0 NO. INFECTED= 1.0 FRACTION=0.333 MEAN WORMS= 0.3 VARIANCE= 0.222 STD.DEV.= 0.471	NO. OF FISH= 12.0 NO. INFECTED= 7.0 FRACTION=0.583 MEAN WORMS= 1.7 VARIANCE= 2.389 STD.DEV.= 1.546
1-30	NO. OF FISH= 2.0 NO. INFECTED= 2.0 FRACTION=1.000 MEAN WORMS= 1.5 VARIANCE= 0.250 STD.DEV.= 0.500	NO. OF FISH= 12.0 NO. INFECTED= 9.0 FRACTION=0.750 MEAN WORMS= 1.4 VARIANCE= 0.910 STD.DEV.= 0.954	NO. OF FISH= 1.0 NO. INFECTED= 1.0 FRACTION=1.000 MEAN WORMS= 1.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 1.0 NO. INFECTED= 0.0 FRACTION=0.0 MEAN WORMS= 0.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 1.0 NO. INFECTED= 1.0 FRACTION=1.000 MEAN WORMS= 3.0 VARIANCE= 0.000 STD.DEV.= 0.000
-35	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS= 2.0 VARIANCE= 0.000 STD.DEV.=******	NO. OF FISH= 17.0 NO. INFECTED= 9.0 FRACTION=0.529 MEAN WORMS= 1.3 VARIANCE= 2.796 STD.DEV.= 1.672	NO. OF FISH= 3.0 NO. INFECTED= 2.0 FRACTION=0.667 MEAN WORMS= 1.7 VARIANCE= 1.556 STD.DEV.= 1.247	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS=***** VARIANCE=****** STD.DEV.=******	NO. OF FISH= 4.0 NO. INFECTED= 3.0 FRACTION=0.750 MEAN WORMS= 1.5 VARIANCE= 1.250 STD.DEV.= 1.118
-40	NO. OF FISH= 1.0 NO. INFECTED= 1.0 FRACTION=1.000 MEAN WORMS= 4.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 6.0 NO. INFECTED= 6.0 FRACTION=1.000 MEAN WORMS= 2.3 VARIANCE= 0.889 STD.DEV.= 0.943	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS=***** VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS=***** VARIANCE=****** STD.DEV.=-0.000	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=0.000 MEAN WORMS= 0.0 VARIANCE= 0.000 STD.DEV.=-0.000
-45	NO. OF FISH= 1.0 NO. INFECTED= 1.0 FRACTION=1.000 MEAN WORMS= 4.0 VARIANCE= 0.0 STD.DEV.= 0.0	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS= 0.1 VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS= 1.1 VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=-.000 MEAN WORMS= -0.0 VARIANCE=****** STD.DEV.=******	NO. OF FISH= 0.0 NO. INFECTED= 0.0 FRACTION=***** MEAN WORMS=***** VARIANCE= 0.000 STD.DEV.=******
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	TOTAL FISH=197.0 INFECTED FISH= 49.0 FRACTION=.421	TOTAL FISH=216.0 INFECTED FISH=118.0 FRACTION=0.546	TOTAL FISH=143.0 INFECTED FISH= 83.0 FRACTION=0.580	TOTAL FISH=120.0 INFECTED FISH= 48.0 FRACTION=0.375	TOTAL FISH=205.0 INFECTED FISH= 40.0 FRACTION=0.195

TABLE 13
INTENSITY OF INFECTION OF FISH WITH
JUVENILE WORMS (Mean Worm Burden)

Size Class cm	June	July	August	September	October
0.0-5	0	0	0	7.8	17.0*
5.1-10	0	0	0	4.8	5.4
10.1-15	0	0	0	0.5	11.2
15.1-20	0	0	0	0.0	0.5

*Indicates a single infection

Analysis of Table 13 indicates that freshwater-drum from 0 through 20 centimeters long harboured juvenile stages and that these stages were present in the fish only in September and October. This correlates exactly with frequency distributions presented earlier. This Table also illustrates that, in general, as the fish increase in size the mean worm burden decreases. These freshwater-drum would be of the 0+, 1+ and 2+ age classes and the 0+ and 1+ age classes carried the greatest mean burden of *Philometra* juveniltes.

Tables 14 and 15 are concerned with incidence of infection with juvenile worms.

TABLE 14

INCIDENCE (PERCENTAGE) OF FISH
INFECTED WITH JUVENILE WORMS

Size Class cm	June	July	August	September	October
0.0-5	0	0	0	83.3%	100.0%*
5.1-10	0	0	0	84.0%	83.0%
10.1-15	0	0	0	23.1%	97.1%
15.1-20	0	0	0	1.8%	14.9%

*Indicates Single Infection

TABLE 15
TOTAL MONTHLY INCIDENCE OF INFE CTION OF ALL
FISH OF ALL SIZE CLASSES FOR 1972, JUVENILE WORMS

June	July	August	September	October
0	0	0	39.8%	61.0%

These two tables indicate that, in general, incidence of infection decreases as the size of freshwater-drum increases, the same size and age classes (0+, 1+) of fish which had the greatest mean worm burden also have the greatest incidence of infection. All three tables (13, 14, 15) revealed a surprising trend, both mean worm burden and incidence of juvenile worms increased in October. This cannot be due to increased feeding upon infected copepods in October for infected copepods could not live this long. It is more likely that this is a result of larvae which were migrating in tissues to the eyes in September which our techniques did not reveal. This must be checked more thoroughly in the coming autumn. The lower figure in Graph 12 summarizes the average percentage of incidence-size class-temporal data for juvenile infections.

Tables 16, 17 and 18 were derived from data present in Table 11.

TABLE 16
MONTHLY INTENSITY OF INFECTION OF FRESHWATER-DRUM
SAMPLES IN 1972. MEAN BURDEN OF ADULT FEMALE WORMS

Size Class* cm	June	July	August	September	October
10.1-15	2.6	1.5	0.4	0	0
15.1-20	0.8	0.5	0.0	0	0
20.1-25	0.1	0.1	0.0	0	0

*All other size classes were negative or had only single infections.

Study of Table 16 indicates that freshwater-drum 10 through 25 cm long harboured the vast majority of adult worms in the months of June, July and the first week of August 1973. After the first week of August

1973 the gravid adult females had streamed from the eyes. This Table also shows that mean intensity if less in July than in June, this would be expected as many adult females penetrate the conjunctiva and stream during July. Fish in these size-ranges would be of 0+, 1+ and 2+ age classes. One can readily ascertain from Table 16 that the smaller fish, 0+ and 1+, age classes are carrying the burden of adult worms, the mean worm burden decreases as the size increases.

Tables 17 and 18 concern monthly incidences of infection of adult female worms.

TABLE 17
MONTHLY INCIDENCE (PERCENTAGE) OF FISH INFECTED WITH ADULT WORMS, FEMALES-1972

Size Class cm	June	July	August	September	October
0.0-5	0	0	0	0	0
5.1-10	0	0	0	0	0
10.1-15	76.5%	63.4%	26.3%**	0	0
15.1-20	35.7%	28.4%	1.4%*	0	0
20.1-25	11.1%	6.7%	4.2%*	0	0

*Indicates a single infection
**Taken during the first week in August 1972

TABLE 18
TOTAL MONTHLY INCIDENCE OF INFECTION OF ALL FISH OF ALL SIZE CLASSES FOR 1972 ADULT FEMALE WORMS

June	July	August	September	October
51.4%	34.3%	8.4%	0	0

These tables correlate well with Table 16. The same sizes and age classes of fish are involved and there is a decrease in incidence as the size of the fish increases. 0+ and 1+ age classes have the highest incidence of infection and the incidence decreases in each size class from June to July to August. Graph 12 summarizes the average incidence-size class-temporal data for adults compared with juveniles.

Encapsulations are given in the same manner in Table 12. No further tables were derived for encapsulations. An inspection of Table 12 shows that encapsulations may be found throughout the entire collecting period as we indicated earlier. This is to be expected as encapsulations persist in the eyes for at least one to two years after infection. The incidence and intensity of infection with encapsulations is greatest in fish 15 cm or more in length, fish which are 1+ age class or older, and in general levels out in fish of the 2+, 3+ or 4+ age classes. These would be fish which were infected in some previous season.

Graph 12 summarizes the average incidence, age class, temporal data for all three phases of *Philometra* sp. in freshwater-drum.

The statistics and analyses given here confirm our opinions given in our reports throughout this year, and they have indicated some problems for further investigation during our work in 1973-74.

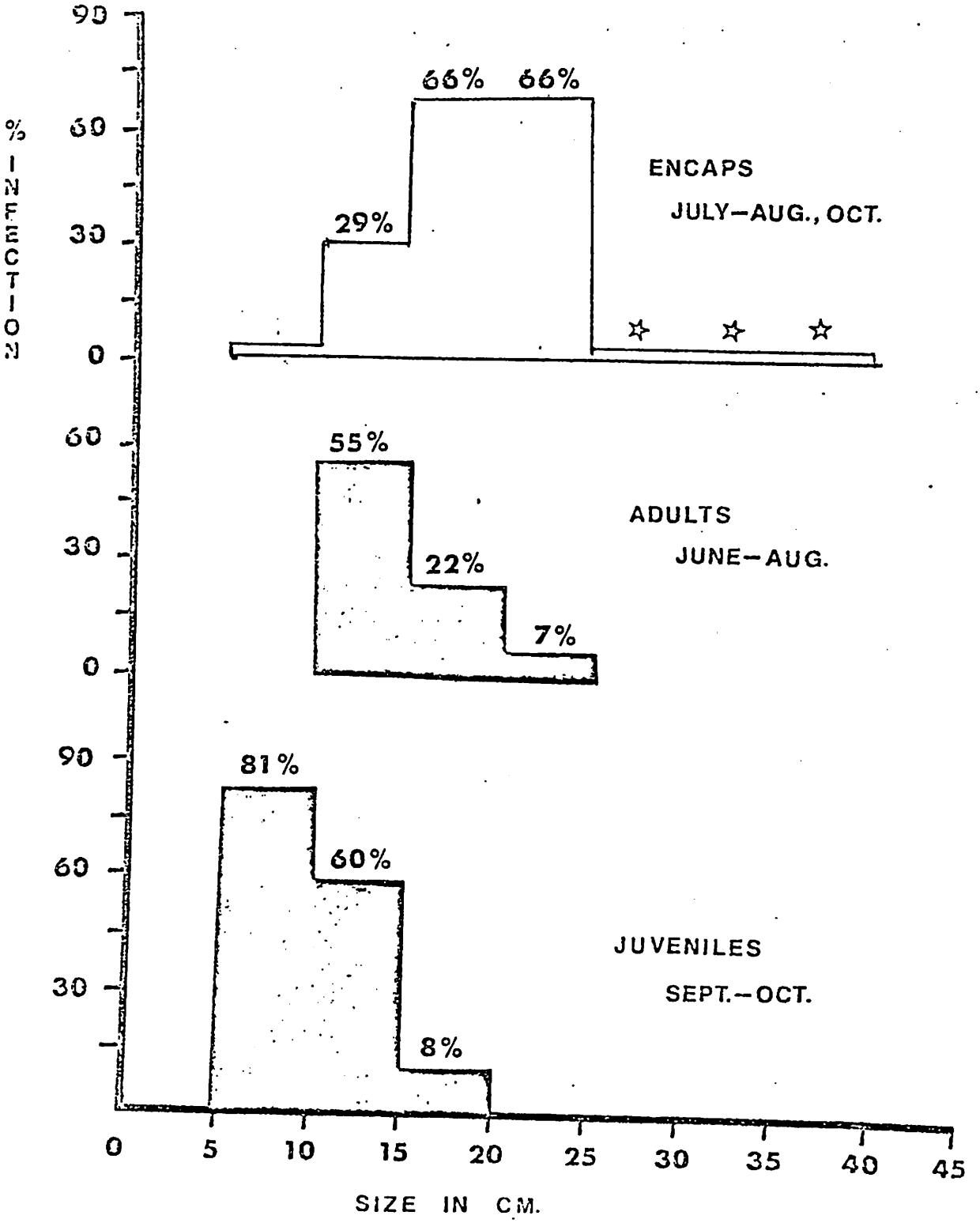
VII. RECOMMENDATIONS

The study has provided a basic framework of data during this first year of operation but much work remains to be done and in several cases more careful and detailed observations, experiments and analyses are warrented.

Better sections through heads and eyes of infected fish are required for studies of histo-pathology and tissue damage. Sections should be made of the eyes of fish exhibiting the :pop-eye" syndrome, containing adult female worms: and through the heads of smaller freshwater-drum with larvae and juvenile stages in the tissues of the eye. Sections should also be

GRAPH 12

% PHILOMETRA INFECTION VS. FISH SIZE



☆ Present but insufficient sample size.

prepared through a series of encapsulations of portions of the streaming female remaining in the eye. The series should be graded from very fresh encapsulations through very old ones. These sections would demonstrate the process of connective tissue envelopment of the worm tissue and tissue reactions of other eye tissues as the partial female worm degenerates.

Besides the information gained from tissue sections experiments should be designed and executed to test living infected sheepshead for impairment of vision or blindness.

One gap in our information which must be filled is the pathway of migration of larvae from the body cavity to the eyes. This migration may also cause some pathological affects not yet recognized in small *Aplodinotus grunniens*.

All nematodes are believed to have four cuticular molts, only two molts have elucidated for *Philometra* sp. as they occurred in the haemocoel of copepods. The times and tissue sites of two further cuticular molts have yet to be determined for stages in the body of the fish host, and the tissue site of sexual diffentiation of male and female worms has not been clearly demonstrated. The timing of all events within the body of the fish needs to be rechecked and verified both in experimentally infected fish and fish from natural populations during the coming year.

The possibility of crustaceans other than copepods acting as intermediate should be carefully checked by infection experiments.

Since parasites sometimes show different morphological characteristics and behavior in different host further cross-infection experiments should be conducted between *Philometra cylindracea* from yellow perch and

Philometra sp. from the eyes of freshwater-drum to assure that these are definitely different species. If these experiments are negative then the species from the eye of the sheepshead should be described in morphological detail and published as a new species.

Philometra cylindracea should be investigated and its transmission cycle in *Perca flavescens* demonstrated, particularly if the cross infection experiments described above are negative, as yet there are no known males for this species and little is known about its distributions in natural populations of fish hosts.

The collection of quantitative data for *Philometra* sp. in the eyes of naturally infected freshwater-drum and the computerized programs should be continued and further statistical testing of the data developed. Data from the natural populations will be more reliable when taken over a period of two more years. At present it is possible to make some predictions based on one year's analysis but accurate predictive modeling is not yet feasible.

The following is an example of a prediction which is of potential use as a recommendation in fisheries management in Lake Erie. If freshwater-drum are shipped alive from western Lake Erie they should be 20 cm or more in length. Fish of more than 20 cm length have only a 3 percent probability of having *Philometra* sp. in their eyes, thus a low probability of spreading the worms in other areas. Caution should rule in this case for this statement is based only on this year's analysis of data and we would prefer to have a larger sample over a period of at least three years before making such a commitment.

Prepared By:_____ Approved By:_____

John L. Crites
Principal Investigator

Research Supervisor
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Date:_____

_____ Approved By:_____

John L. Crites
Project Leader

Date:_____ Date:_____

STATE: OHIO

PROJECT NO: F-48-R-1 PROJECT TITLE: Impact of parasitic "red worms" on Lake Erie fishes

STUDY NO: II STUDY TITLE: Impact of *Camallanus oxycephalus* on Lake Erie fishes

PERIOD COVERED: June 1, 1972-May 31, 1973

I. SUMMARY

The study of the impact of the nematode *Camallanus oxycephalus* on Lake Erie fish during 1972-1973 centered around description of the parasite and the elucidation of the dynamics of transmission. The development and transmission pathways were studied in the laboratory as well as in the western end of Lake Erie. In addition, the extent of infection in the fish com munity was analyzed. A series of experiments designed to study the biology of the dispersal agent were carried out. Information concerning population flucuations of the parasite was collected to be incorporated into a model at a later date.

II. CONTENTS

III. BACKGROUND

Both sportsmen and commercial fishermen have complained for years about a large, red worm which protrudes from the anus of fish in Lake Erie. Fish exhibiting this syndrome are unsightly and discourage sportsmen. A preliminary investigation of this problem revealed that this "Redworm" is the nematode, *Camallanus oxycephalus*. It has been reported from 26 species of fish in western Lake Erie. In some species, such as white bass, and crappies, infections are heavy and close to 100% of the population is infected during some seasons. Comparison of our preliminary findings on white bass, the most frequently and heavily infected species, with previous studies on Lake Erie fish parasites in 1927 and 1957, revealed that the incidence of *Camallanus* has doubled. In addition, our data suggested that the intensity of infection increased as well.

Our broad objectives in this study were to assess the impact of *Camallanus* on the affected fish populations. Because white bass were common and heavily infected, we directed our research effort at this species to gather information about pathology, disease, transmission, factors affecting transmission and population ecology of the parasite. In addition we planned to survey other fish populations for *Camallanus* to determine how the incidence has changed and perhaps what changes in Lake Erie have contributed to that.

The information generated by this investigation will hopefully be useful to the management and control of this parasite in Lake Erie as well as in fish-out ponds which are stocked with Lake Erie fish.

IV. OBJECTIVES

There were three jobs for this study, each with several, specific objectives. Our first objective was to acurately describe the parasite, to insure identification. No complete description of *C. oxycephalus* was found anywhere in the literature. Our second job was broadly defined as experimental investigation. Under this job our objectives were twofold: 1) To determine how *Camallanus* develops and is transmitted; and 2) What environmental factors affect the development and transmission. The third job objective was to study the population biology of *Camallanus*. We were specifically interested in elucidating any population regulatory mechanisms which might be useful in management. In addition, we hope to develop a general model of population flucuations with the hope of predicting parasite levels.

V. PROCEDURES

During the first quarter of the project, the parasitological laboratory was moved to Franz Theodore Stone Laboratory at Put-in-Bay. Fish were collected by otter trawl, gill net, fyke net, seine and hook and line. All samples were placed on ice and examined fresh. Young of the year fish were collected by seine and dip net and maintained in laboratory holding tanks. Invertebrates for experimental work were maintained in laboratory culture. Larvae of *Camallanus* were obtained from gravid female worms and stored in constant temperature cabinets for experiments. Fish were anesthetized with Quinaldine and infected orally with a stomach tube. Development of the worms was studied at intervals following infection.

Population data was gathered by sampling white bass, yellow perch and freshwater drum bi-weekly. In addition, plankton samples were taken with a 3 liter Kemmerer water bottle three times each week.

During the second, third and fourth quarters, the project worked out of the parasitological laboratory at The Ohio State University in Columbus. Fish were sampled at least once per month except during the winter months. Worms were measured and studied and data from the experimental and population investigations were keypunched on computer cards. Statistical analyses were carried out in FORTRAN IV on an IBM 370/165 digital computer.

VI. FINDINGS

Infection and Development: First stage larvae of *Camallanus* were fed to numerous different aquatic arthropods, but only copepods were infected (Table 1). Cyclopoid copepods feed upon the larvae much more readily than calanoid copepods. Development within the copepod hemocoel was rapid at 25°C. The first molt occurred at 3 days post infection, the second molt occurred at 6-7 days p.i. Development at 20° and 15°C was slower, the third stage being reached at 10 and 26 days (Figure 1) respectively. These third stage larvae are infective to fish immediately. All larval stages and adults were measured, described completely and figured.

When infected copepods were fed to fish, bile stimulated the third stage larvae to excape from the copepod carcass in the fish stomach or small intestine. The third molt occurred on the 9-10 day p.i. at 26°C. The final molt occurred at 18 days p.i. for male worms and 24 days for female (Figure 2).

TABLE 1

EXPERIMENTAL DETERMINATION OF INTERMEDIATE HOST FOR *C. OXYCEPHALUS*

Potential Intermediate Host	Larvae Eaten	Larvae Penetrated
Gammarus	+	-
Hyallela	+	-
Asellus	+	-
Cyclops	+	+
Diaptomus	+	+
Bosmina	-	-
Daphnia	-	-
Stenonema	-	-
Chironomus	+	-
Cricotopus	+	-

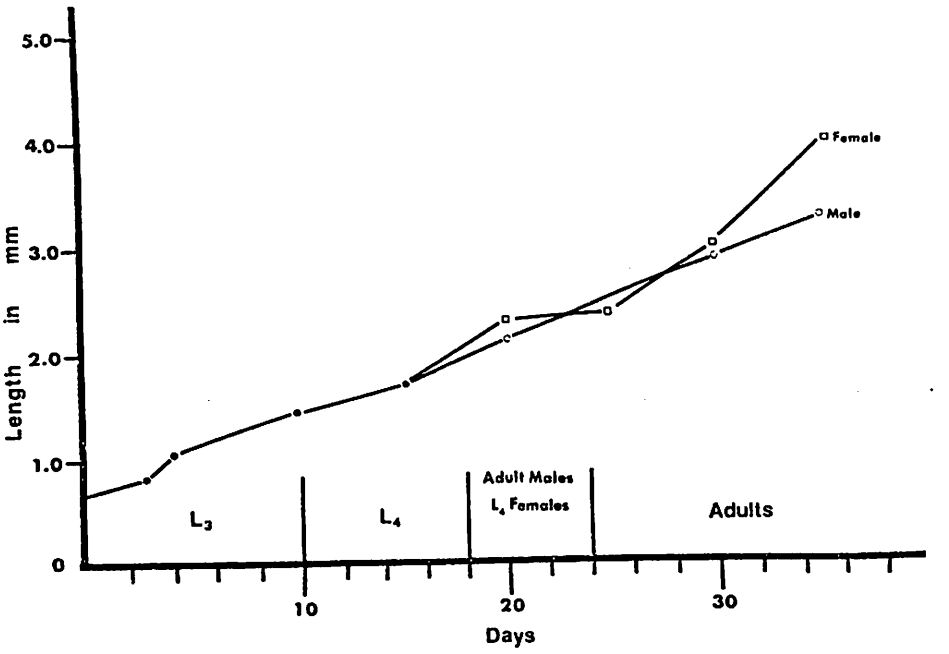


FIGURE 1
FINAL DEVELOPMENT OF *C. oxycephalus* IN FISHES

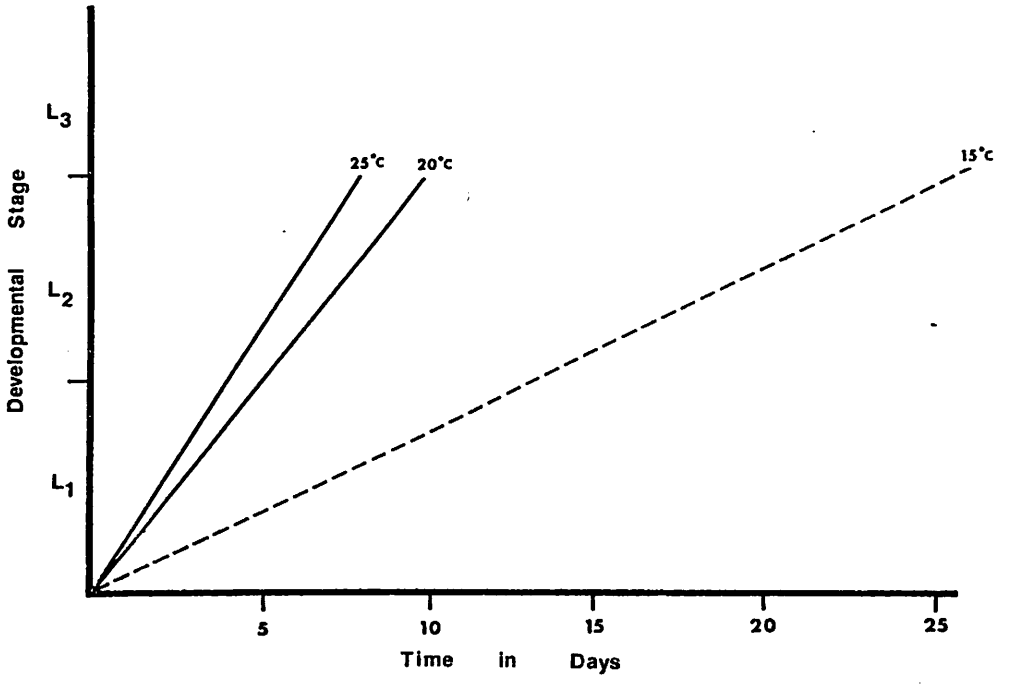


FIGURE 2
EFFECT OF TEMPERATURE ON DEVELOPMENT OF L_3 OF *C. oxycephalus*

The experiments revealed that the infection can be transmitted to fish directly from copepods. However, when small, infected forage fish were fed to young perch and the nematodes were transferred and developed. Thus, transmission of this parasite has two routes in Lake Erie: 1) A direct route from copepods to planktivorus fishes and 2) an alternate from infected planktivorous fishes to larger predatory species (Figure 3 and 4).

Studies on the Infective Larvae: Experiments on the survivorship (Figures 5-7) , activity and infectivity were conducted at 20° and 25°C. These indicated that the larvae live for a much shorter time at 25° than 20°. In addition, their ability to infect the copepod is lost prior to death. The infectivity decreased more rapidly at 25°C. Active movements of the larvae decreased linearly with age, but infectivity decreased logrithmically, suggesting that penetration of the copepod gut wall is not entirely a mechanical process.

A survey of Lake Erie fish for *Camallanus* revealed that 15 species were found to be infected. Comparison of our findings with surveys done in 1927 and 1957 showed that the incidence of the parasite increased in 10 species, remained unchanged in six and decreased in one (Table 3). Most of the species which have become more frequently infected are piscivorous, suggesting an increased flow of the parasite through the forage fish populations. A paricularly significant finding was the infection of gizzard shad and alewife with *Camallanus*. We believe that the increase of *Camallanus* in Lake Erie is related to the increase in abundance of the clupeids which are frequently infected.

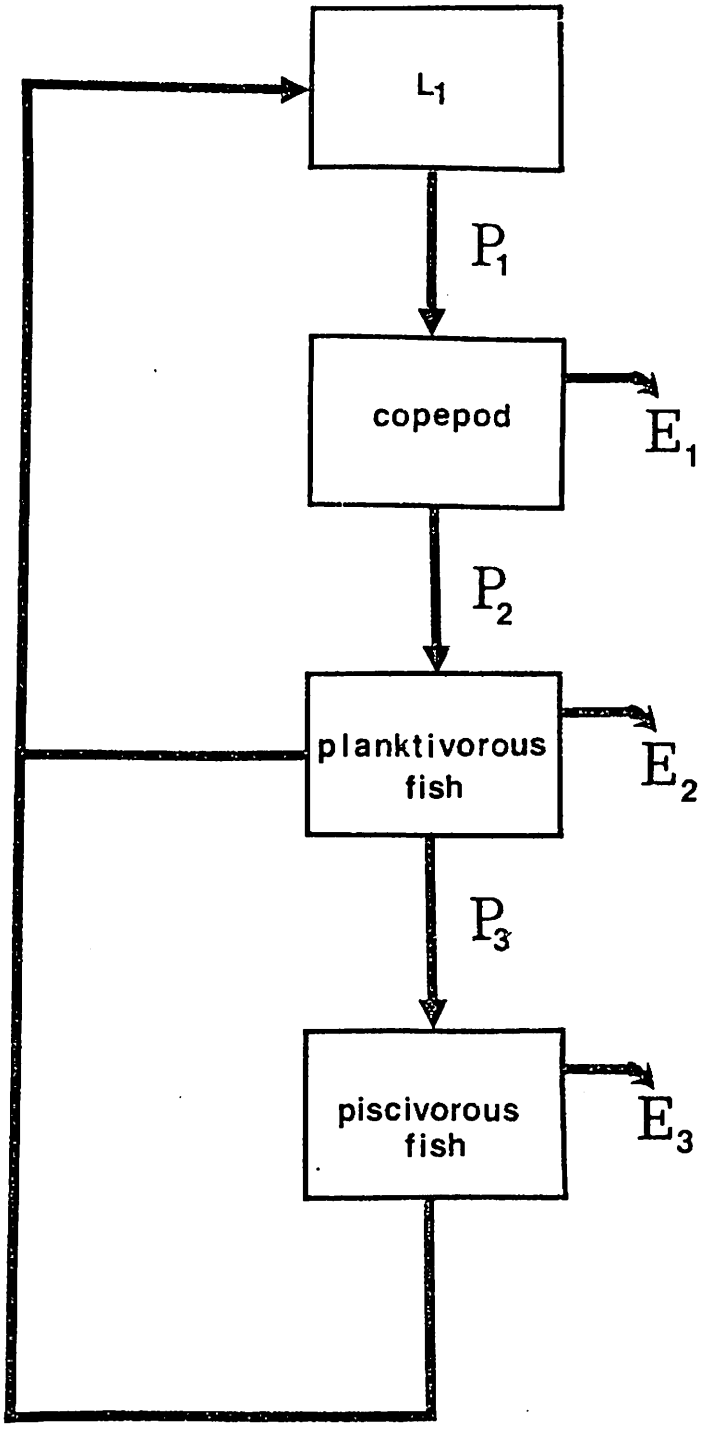
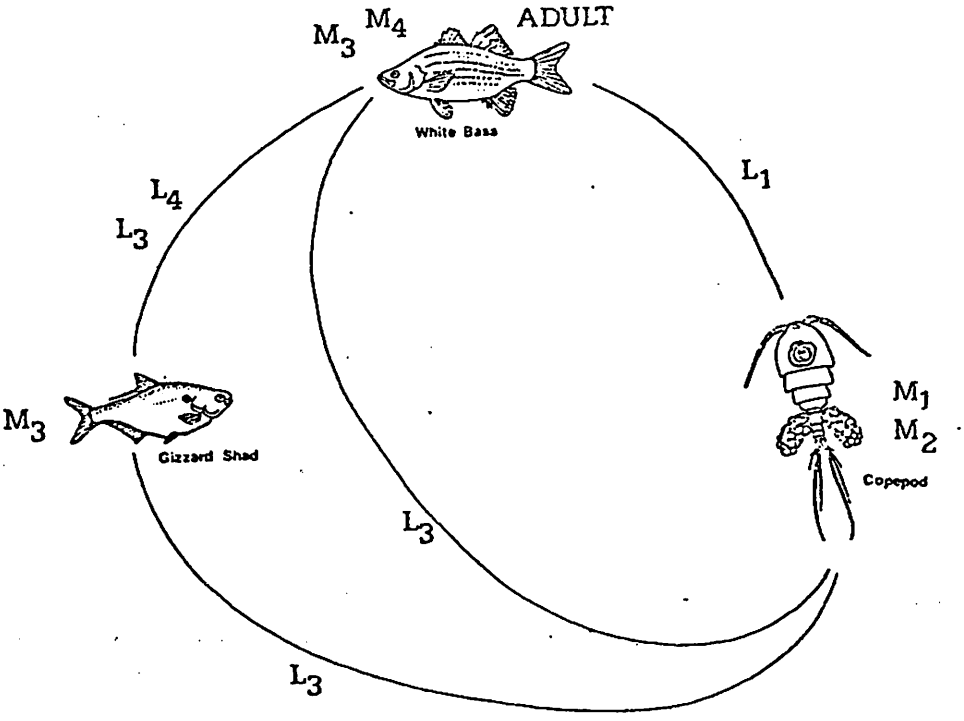


FIGURE 3
LAKE ERIE-*Camallanus* SYSTEM
Px=Probability of contact
Ex=Efficiency of transfer



Life History of Camallanus oxycephalus

FIGURE 4

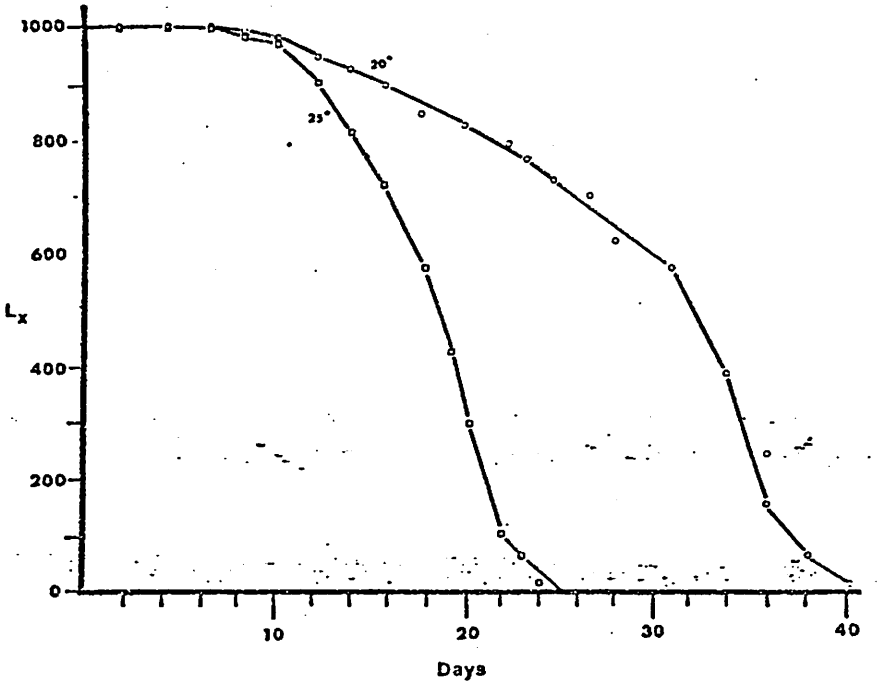


FIGURE 5

SURVIRORSHIP OF *C. oxycephalus* 1st STAGE LARVAE

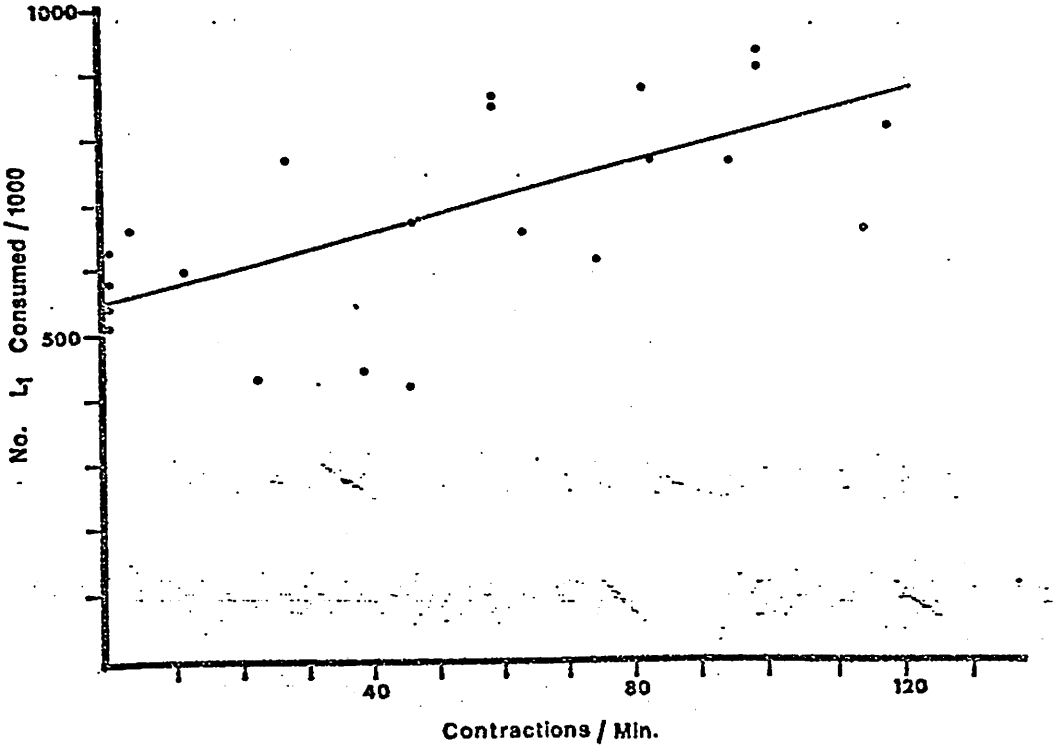


FIGURE 6
ACTIVITY OF 1st STAGE LARVAE OF *C. oxycephalus*

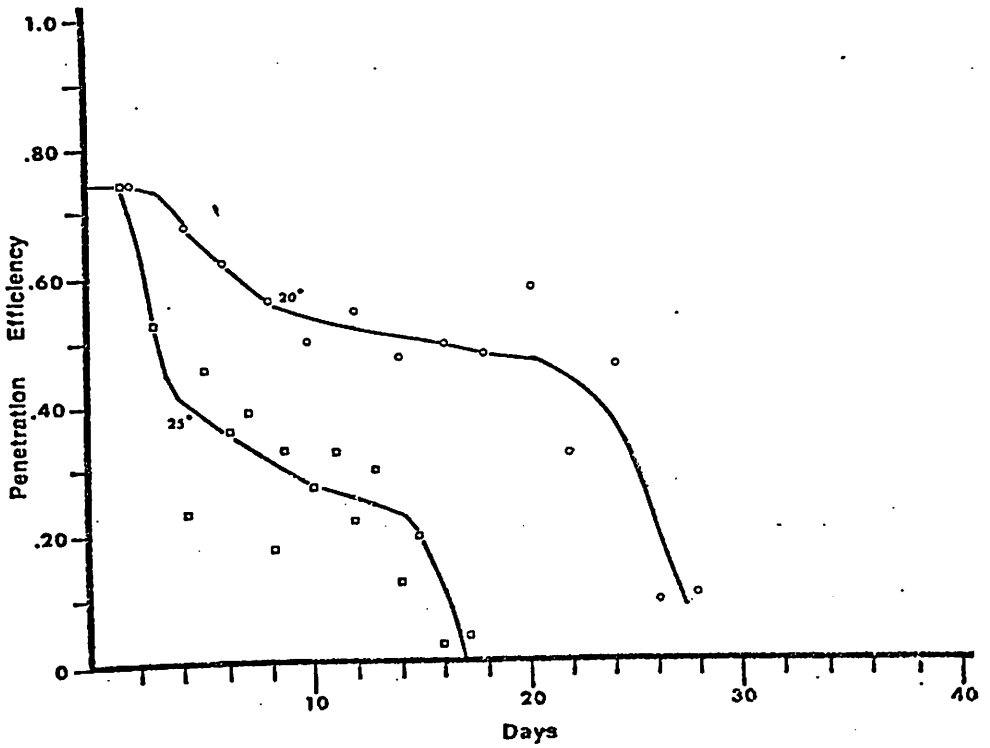


FIGURE 7
INFECTIVITY OF 1st STAGE LARVAE OF *C. oxycephalus*

TABLE 3

Comparison of Frequency of Camallanus oxycephalus in Lake Erie Fishes

Fish	1927		1957		1972		X^2
	F	N	F	N	F	N	
Morone chrysops (Adult)	.469	32	.472	53	.952	83	8.25**
M. chrysops (YOY)	.220	9	-	-	.641	170	
Aplodinotus grunniens	.400	45	.143	88	.507	67	9.99**
A. grunniens (YOY)	-	-	-	-	.500	300	
Perca flavescens (Adult)	.022	45	.054	93	.475	114	303.02**
P. flavescens (YOY)	.000	15	-	-	.016	64	
Pomoxis annularis	.231	17	.396	53	.708	48	7.69**
P. nigromaculatus	.111	9	.310	29	.730	37	8.87**
Ambloplites rupestris	.116	12	.107	75	.273	22	3.82
Lepomis spp.	.061	33	.194	144	.107	28	3.16
Micropterus dolomieui	.125	80	.078	55	.375	40	13.29**
M. salmoides	.023	129	.175	40	-	-	11.70**
Stizostedion spp.	.104	48	.212	33	-	-	1.62
Etheostoma/Percina	.161	93	.102	127	-	-	1.48
Percopsis omiscomaycus	.369	69	.032	63	.077	13	11.29**
Ictalurus spp.	.034	29	.026	39	.167	57	6.00
Notropis spp.	.055	274	.017	287	.027	264	7.22
Osmerus mordax	-	-	.000	61	.040	50	
Dorosoma cepedianum	-	-	.000	27	.533	360	
Alosa pseudoharengus	-	-	.000	14	.121	190	

** Denotes significant X^2 value. F = Frequency of Infection. N = Number of fish examined.

Population Biology: Studies on the population biology of *Camallanus* revealed that the worms live for one year. They released their larvae and died during late June to late July. This period coincided with the annual maximum density period of the intermediate host. Infection of fishes occurred during August. The worms grew until November, when growth was arrested. Growth did not begin again until mid-April. At this time, growth was very rapid and eggs and larvae began to appear in the female uterus (Figure 8).

Infections in adult white bass were very heavy during August but dropped rapidly during autumn. Infections in young of the year white bass were not as heavy and no drop in the worm population occurred. This lowering of worm population was either produced by a density-dependent parasite population regulation or death of heavily infected fish.

VII. RECOMMENDATIONS

Further studies on environmental factors affecting the 1st stage larvae are indicated. Until more information is available, management must be concerned with controlling the spread of this parasite. Removal of fish from Lake Erie to fish-out ponds should be carried out during the period of minimum infection. This period extends from late June to late July. In addition, it is recommended that the use of gizzard shad and alewife should be avoided if possible in those bodies of water to be stocked with Lake Erie white bass.

Studies on the population biology will be continued. We hope to develop some additional information concerning the population regulation and stability. In addition, we intend to investigate the pathology caused by the parasite.

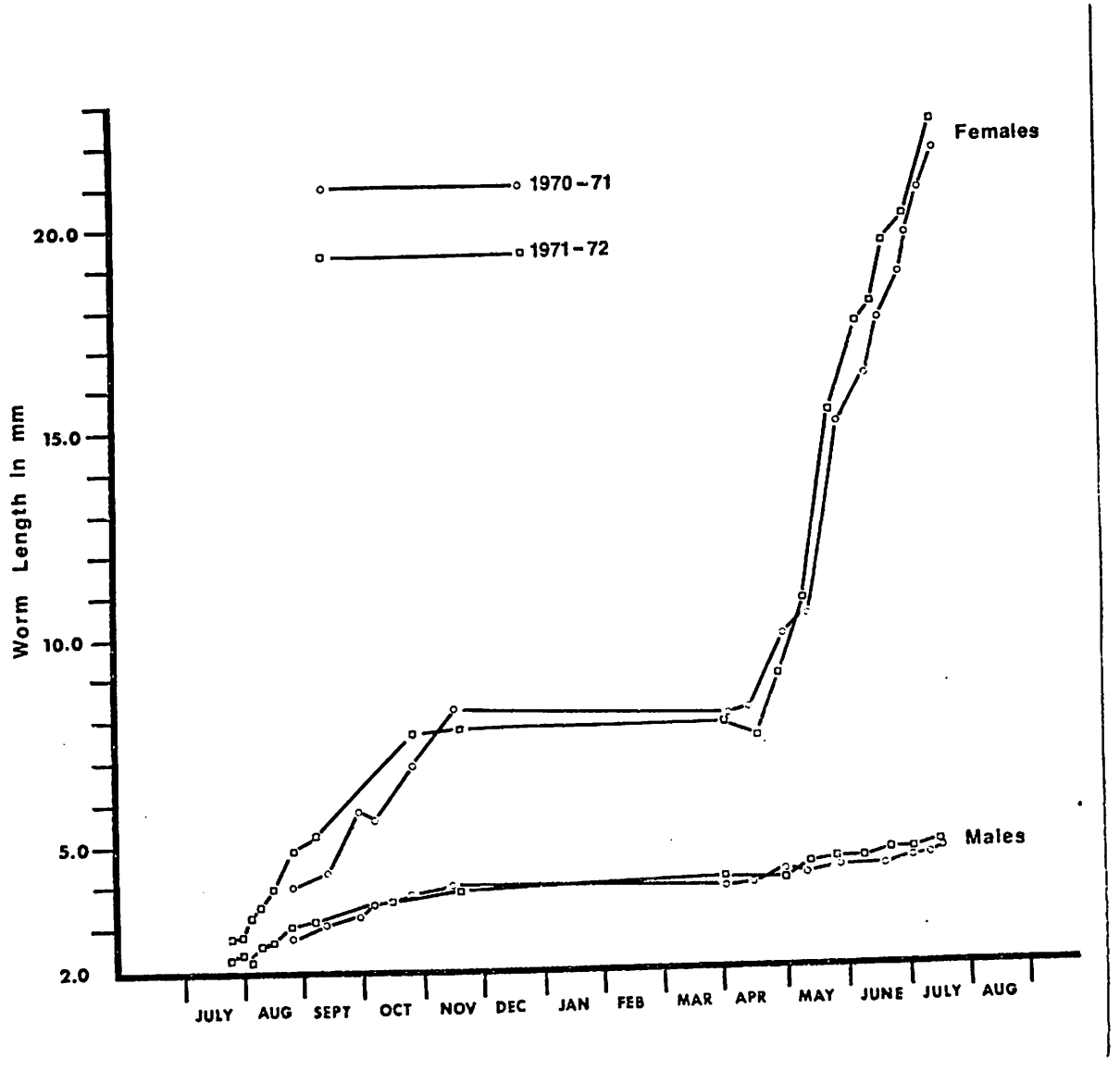


FIGURE 8
NATURAL GROWTH CURVES FOR ADULT *C. oxycephalus* IN *Morone chrysops*

Publications from this work include a Ph.D. dissertation, a paper describing the species, a paper on the analysis of the changes in the incidence of infection and a paper describing the life cycle. In addition, abstracts were submitted and papers presented to the 47th and 48th Annual Meetings of the American Society of Parasitologists, the International Association of Great Lakes Research, The Ohio Academy of Science and the Wildlife Disease Association.

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PROJECT NO: F-48-R-1 PROJECT TITLE: Impact of parasitic "red worms" on Lake Erie fishes

STUDY NO: III STUDY TITLE: Impact of *Eustrongylides tubifex* on Lake Erie fishes

PERIOD COVERED: June 1, 1972-May 31, 1973

I. SUMMARY

Several factors were elucidated pertaining to the transmission cycle of the parasitic nematode, *Eustrongylides tubifex* in the Western Basin of Lake Erie. Larval forms, third and fourth-stage larvae, are recovered from the fish intermediate host, *Perca flavewcens*. The smaller larvae were found in small encapsulations adhered to the intestinal wall, lying in the mesentery, or the larvae were free in the mesentery. The larger, fourth-stage larvae were usually recovered from yellowish-brown mesentery encapsulations in the body cavity. Mostly third-stage larvae, very few fourth-stage larvae,were recovered from freshwater-drum, *Aplodinotus grunniens*. It is concluded that the majority of larval *E. tubifex* are not well adapted to developing in drum as compared to perch.

Fourth-stage larval *E. tubifex* from yellow perch were found to be invasive by experimental infections for the following birds: mallards, *Anas platyrhynchos;* black-crown night herons, *Nycticorax n. hoactili;* and ring-neck pheasants, *Phasianus colchicus torquatus*. The larval *E. tubifex* were quite detrimental to the pheasants and the nestling night heron. One pheasant and the night heron succombed to verminous peritonitis. Pheasants, definite "abnormal" bird hosts, were susceptible to larval

migrations through the body of the host. In both the heron and the pheasants, the larvae failed to develop or become encapsulated but were encountered either actively burrowing through the flesh and viscera or degenerating.

The development of larval *E. tubifex* to maturity was completed with the voidance of fertilized eggs in mallards only. From the literature the definitive hosts for *E. tubifex* were listed as being mainly fish-eating ducks: mergansers, mallards, loons, murres, auks, and cormorants. From our investigations adult *E. tubifex* were recovered from the experimental mallard infections and from a natural infection of a red-breasted merganser, *Mergus serrator*. Adult *E. tubifex* were encountered from the mallard infections 7-14 days *post infectionem* . Males underwent their last molt 7-9 days *post infectionem*. After 16 days *post infectionem*, all males recovered were in a state of degeneration. Females molted 7-16 days *post infectionem*.

Infection experiments with nestling herring gulls, *Larvus argentatus*, were negative. In autopsies of frozen proventriculi from great blue herons, *Ardea herodias*, and merican egrets, *Casmerodius albus egretta*, no signs of natural infection with *Eustrongylides* were noted. However, a few black-crown night herons, *Nycticorax n. hoactli*, 3 adults and 1 nestling, were naturally infected with fourth-stage larval *Eustrongylides tubifex*.

A description of the third and fourth-stage larvae was written from the larval *E. tubifex* recovered from *Perca flavescens*. Molting appears to be a complex process in the life cycle of *E. tubifex*. No distinct molt was observed between the arbitrarily designated third and fourth-stage larvae in the fish host *Perca flavescens*. Upon investigation of the morphology of the larvae, it seemed plausible to separate them into two

distinct morphological groups similar to those reported by Karmanova (1968) for *E. excisus*. Whereas the larvae designated third-stage have one cuticle, the larvae designated fourth-stage are acquiring a double cuticle toward the end of their size range, the characters of the adult seen within the last cuticle. The molting process and morphology of these larvae require further study.

After study of the adult forms recovered from mallard experimental infections and comparison with specimens of the U. S. Museum Helminthological Collection, the species was established as *E. tubifex*, the type species of the genus. The distribution of this species is thus expanded from Western Europe and the U.S.S.R. to the U.S.A. Added to the species heretofor encountered in the literature for U.S.A., *E. ignotus* and *E. mergorum*, is thus the type species *E. tubifex*. Key characters used to elucidate the species *E. tubifex* were (1) 12 papillae located around the mouth, (2) the lack of a deep cleft in the male bursa, (3) the outer circle papillae much larger than the inner circle papillae, (4) the male bursa with a fringed cuticular border, and (5) the inner papillae terminating wtih straight spine-like structures. Redescriptions of the adult male and female were written based on specimens recovered from mallard infections.

A description of the pathology to the fish and bird hosts was written. The adult *E. tubifex* in mallards are entwined within thick connective tissue encapsulations in the form of coiled tubes on the serosal side of the proventriculus. Larval *E. tubifex* were predominantly localized in pulpy to granular encapsulations in the mesentery or fatty tissue in the

body cavity of the fish. Occasional pulpy lesions were noted near the encapsulations. The encapsulations consist of a hyperplasia of connective tissue in which are trapped pieces of pancreatic tissue. The pancreatic tissue nearer the periphery of the capsule appears normal, while that more deeply trapped appears abnormal and resembles liver tissue.

From our population studies of the level of eustrongylidosis in fish and birds in the Lake Erie region a few conclusions can be made. It appears that most of the larval *E. tubifex* are not well adapted for developing in the freshwater-drum, *Aplodinotus grunniens*. The majority of *E. tubifex* recovered are hardened, dead encapsulations of third-stage larvae.

In yellow perch, *Perca flavescens*, development from third to fourth-stage larval *E. tubifex* apparently readily occurs. Very few dead or degenerating larvae were recovered. The older and larger the perch, the higher the level of infection. Transmission from the probable first intermediate host, freshwater oligochaetes, to perch would have a higher probability with the older, larger perch. An accumulation of the worms in the fish host through the life of the fish is indicated by an increase in the mean number of worms of infected fish with age and the wider ranges of worms in the older fish.

The tissue sites most often selected by third stage larval *E. tubifex* are small pink capsules on or near the intestinal wall or free in the mesentery. Fourth-stage larvae are most often encountered in yellowish-brown granular encapsulations in the mesentery or fatty tissue in the body cavity of the fish. Those larvae encountered burrowing in the musculature or viscera, I believe, are responding to some sort of stress. Possible

stress factors include an increase in temperature, degeneration of the perch flesh after capture before autopsy, and physical stress to the fish.

No definite seasonal pulse in infection levels could be detected. It appears then that perch are acquiring the infection throughout our collection period.

Preliminary investigations indicate that natural bird hosts acquiring eustrongylidosis in the Lake Erie region are black-crown night herons and red-breasted mergansers. However only larval forms of *E. tubifex* were encountered in the black-crown night herons whereas the merganser harbored an adult infection.

II. CONTENTS

III. BACKGROUND

The maintenance of the parasitic worm *Eustrongylides tubifex* population in nature appears to be comple in nature. A definitive bird host, a fish intermediate host, plus a first intermediate host play essential roles in the transmission of the worm. We assume fish are necessary intermediate agents of transmission since most of the reported bird hosts are aquatic fish-eating birds. Though we do not know how fish acquire infection, we suspect by ingestion of infected freshwater oligochaetes from the investigations of Karmanova (1965) in Russia.

Large red worms, larval *E. tubifex*, have captured the attention and interest of sports and commercial fishermen. The unsightly appearance of the worms in the fillets and body cavity of yellow perch, *Perca flavescens*, has stirred interest in the biology of the worm and its affect on its hosts.

Bangham (1972) states that *Eustrongylides* sp., a tissue invading nematode found in the body cavity and flesh of its host is a seasonal economic factor in that its presence causes rejection of yellow perch fillets Dechtiar (1972) in citing new host records for Lake Erie fish considered *Eustrongylides* sp. pathogenic to fish. Dogiel, Petrushevski, and Polyanski (1961) state that larval *Eustrongylides* sp. parasitic in fish cause a hyperplasia of connective tissue resulting in the encapsulation of the larvae in somewhat round, flattened capsules.

Besides pathogenicity to fish, Karmanova (1968) considered such larval forms capable of inflicting injury to fish eating animals. Bowdish (1948) reported the mortality of a Newark, N. J. great blue heron,

Ardea herodias, from verminous peritonitis. The heron had severe perforations and adhesions of the intestines caused by several young worms tentively identified as *Eustrongylides ignotus*. Locke (1961) records a second case of fatal verminous peritonitis in the great blue heron plus a first case reported for the American egret, *Casmeroduis albus egretta*. In both cases a large shield-like mass of coiled fibrous tubes containing the large red *E. ignotus*, adhered intestines, and ingesta filled the ventral portion of the abdominal cavity. A die-off of red-breasted mergansers, *Mergus serrator*, at Lake Holly, Virginia Beach was attributed to massive tissue destruction and hemorrhage produced by larval *Eustrongylides* sp. migrations (Locke, 1964). Subsequent investigations revealed larval *Eustrongylides* sp. in mosquitofish and silversides upon which the mergansers had been feeding. Shillinger (1936) attributed extensive loss of waterfowl in British Columbia to the presence of great numbers of *Eustrongylides mergorum* in the walls of the proventriculus. Lichtenfels (1973, personal communication) stated that a case of a man coughing up a subadult *Eustrongylides* had been reported and would soon be published.

The numerous findings of *Eustrongylides* sp. reported in the literature from many different species of fish, some amphibians, and a few reptiles plus reports of adult infections in different aquatic birds and subadults from mammals and now man reflects the generalized parasitism of the worm. Problems arise in designating the reservoir hosts maintaining the infection in nature and the accidental hosts picking up the infection but not transmitting it. It appears likely that the more "abnormal" hosts acquiring the infection of the worm exhibit the more severe pathology.

In this study we are aiming our efforts at four project objectives. First we wish to understand the transmission cycle of the worm. In other words, what hosts are involved in maintaining the worm population and the methods of transmission. An understanding of accidental host involvement is also necessary. Besides the transmission cycle, we are gathering information on the level of infection of the worm in the bird hosts, fish hosts, and hopefully the as yet unknown first intermediate host. Analysis of this population data will be in terms of mean worm burdens, relative abundance (% infection), rangesof infection, variance, frequency distributions, and seasonal fluctuations. We also are investigating host pathology, the pathology of the worm in the fish, the bird, and perhaps mammals.

In the course of our investigations a description of the worm morphology is of necessity. This is essential for proper identification, redescription of the species if needed, literature comparisons, and establishment of the geographical distribution.

IV. OBJECTIVES

Job No. III-a: Experimental Phase

1. Determination of the transmission cycle of *Eustrongylides tubifex*.
2. Demonstration of what intermediate hosts are involved in the process of transmission.
3. Demonstration of how and when the infective stages are passed from one definitive host to another.
4. Demonstration of the sequence and times of development of the stages in the different hosts involved.

Job No. III-b: Descriptive Phase

1. Description of the parasitic stages of *E. tubifex* involved in the different hosts.

2. Description and demonstration of routes of migration through the organs and tissues of the hosts and the resultant pathology.

Job No. III-c: Population Phase

1. Determination of the incidence and intensity of infection of *Eustrongylides tubifex* and the effects on natural populations of fish hosts in western Lake Erie.

V. PROCEDURES

Yellow perch, *Perca flavescens*, utilized in this study were collected from two sites in Western Lake Erie. One site was Sandusky Bay near the mouth of Cold Creek on the south shore of Lake Erie, the second was the open lake between Rattlesnake Island and Green Island. At the first site fish were furnished by commercial fish corporations using a 150-foot bag shore seine in the Sandusky Bay area. The fish were placed in ice chests and transported to South Bass Island for autopsy. At the second site fish were collected by otter trawl from the Bio-Lab off Stone Laboratory. After trawling, the fish were quickly placed in ice chests and taken back to the Research Laboratory for autopsy.

Autopsy technique for perch was carried out in two manners, depending on the length of the fish. Young-of-the-year perch, under 10 cm in lenth, were subjected to pepsin digest of the gut and viscera plus the mesentery tissue. The pepsin digest solution consisted of 4 grams of pepsin, 7 ml hydrochloric acid, and 1000 ml of distilled water. After a quick visual check of the body cavity of the young perch for signs of *Eustrongylides*

infection, the mesentery tissue was carefully teased away from the digestive tract and visceral organs. The mesentery tissues were placed into one series of test tubes which were three-quarters filled with freshly prepared pepsin digest fluid. Into a separate series were placed the digestive tract and visceral organs. The test tubes were manually agitated and placed in a constant temperature chamber at 25°C. The tubes were agitated again after 2-4 hours and the contents allowed to settle for approximately 20 minutes. The supernatant was then aspirated off and the digestive fluid replenished. After another 4-8 hours the contents were again agitated, allowed to settle, supernatant aspirated off and Ringer's cold solution was added to the residue. The contents were placed in a glass petri dish and examined using a binocular dissecting microscope utilizing both transmitted and reflected light.

The autopsy procedure for all fish can be summarized as follows: (1) measurement of fish total length in cm., (2) notation of fish sex, (3) pepsin-digest procedure for fish less than 10 cm. in length, and (4) careful dissection of fish greater than 10 cm. in length.

Dissection of perch greater than 10 cm. long consisted of first visually searching the mesentery tissue for signs of yellow granular capsules. When found the larvae were plucked out with a needle point and placed in Ringer's cold solution. Next all mesentery tissue was removed to a glass petri dish with Ringer's cold solution and examined under a binocular dissecting scope. The intestine, stomach, heart, liver and gonads were examined in a like manner. After examination of the viscera,

the body wall and gas bladder were checked for signs of burrowing larvae or pulpy lesions. Finally the external surface of the fish was also examined for migrating red larval nematodes or local lesions. Light transmitted from a bright desk lamp through the fish body wall aided such fidnings of burrowing larvae. The larvae of *E. tubifex* were always placed in petri dishes of Ringer's cold solution after removal from the fish. If larvae were to be used in later infection experiments the covered dishes were placed in a 15°C constant temperature chamber, otherwise the larvae were relaxed and killed in steaming Ringer's cold solution and fixed in alcohol-formalin-acetic acid (AFA) solution for 24 hours, then transferred to 75% ethyl alcohol until later study.

Eustrongylides tubifex larvae were studied for descriptive purposes by clearing the worms in stendor dishes containing an alcohol-glycerine solution, 3 parts 75% alcohol to 1 part glycerine. The stendor dishes were placed in a dessicator and the alcohol evaporated slowly leaving specimens in glycerine. If necessary more glycerine was added to each dish until the nematodes had cleared sufficiently for study. The specimens were then mounted in glycerin on slides under coverslips.

Serial cross sections of larval *Eustrongylides tubifex* specimens were cut with a microtome at 10u thickness and stained with eosin and also by Mallory's technique. The worms were fixed in Bouin-Dubascq Fixative. Also sectioned and stained in eosin, hematoxylin, and by Mallory's technique were the mesentery capsules containing the larval *Eustrongylides tubifex* taken from yellow perch. These slides were sent to the Eastern

Fish Disease Laboratory in Kearnysville, West Virgina for diagnosis of the pathology involved.

Analysis of the population data of infection levels of *Eustrongylides tubifex* in *Perca flavescens* was carried out by key punching the data onto computer cards and then running appropriate computer programs at The Ohio State University's Instruction and Research Computer Center. OMNITAB, FORTRAN, SPSS, and PSTAT were utilized as languages for the various programs. We utilized the programs to obtain cross tabulations, scatter diagrams, and statistical analyses.

Four different species of birds were used in the experimental infections with larval *Eustrongylides tubifex*. The main experimental host was the mallard, *Anas platyrhynchos*. The mallards were second generation fliers from wild mallards purchased from Whistling Wings, Box 1, Hanover, Illinois, 61041 and flown via air express to Port Columbus. The mallards purchased are fed a commercial starter feed with a high protein content before shipment to prospective buyers. The summer of 1971, 15 mallards were used as experimental hosts; the summer of 1972,14. The age of the mallards at the time of infection ranged from 3 to 11 weeks old. The mallards were kept in framed hardware cloth cages on South Bass Island on the premises of Franz Stone Laboratory. The birds were initially fed Purina chick starter and later Purina cracked corn and turkey pellets. Water was obtained from a well on South Bass Island. Ring-neck pheasants, *Phasianus colchicus torquatus*, were utilized as experimental hosts. The young pheasants were obtained from the Department of Wildlife and raised from birth on Purina Chick Starter. Five of these young pheasants were inoculated with *E. tubifex* larvae the summer of 1971. A single nestling black-crowned

night heron, *Nycticorax nycticorax hoactli*, obtained from East Sister Island, Lake Erie, was also an experimental host. The heron was frequently fed coho salmon fry from the State Fish hatchery on South Bass Island and uninfected flesh from *Perca flavescens* and *Morone chrysops*. Purina chick starter also played a part in the diet of the heron. The fourth bird species experimentally infected was the herring gull, *Larus argentatus*. Four young gull chicks ranging in age from 1-2 weeks were obtained from Big Chicken Island, Lake Erie. These gulls were taught to eat moistened Purina chick starter.

Three mallards and two ring-neck pheasants were autopsied as controls the summer of 1971. None of the controls were infected with *Eustrongylides*. Two gull chicks were also autopsied as controls, but none were infected. None of the experimentally infected gulls proved positive either.

Also checked in 1971 were two young black-crowned night herons from East Sister Island; one approximately 1-2 weeks old was found dead and the other 1-2 days old died after capture. Both of these nestling herons were negative to *Eustrongylides* infection. However a regurgitation of a perch by a third nestling *N. nycricorax hoactli* at East Sister Island revealed an actively burrowing *Eustrongylides* larva in the flesh of the fish.

Only the large red larval *Eustrongylides tubifex* (fourth-stage larvae) were used for infection purposes. These larvae were stored in covered petri dishes in Ringer's cold solution. The summer of 1971 they were kept in a refrigerator, while the summer of 1972 a 15°C constant temperature cham er proved more advantageous. The time ofstorage from extraction of the larvae from the fish host to inoculation into a bird ranged from a

few hours up to twelve hours. It was observed that larvae could remain viable 3-5 days in a 15°C constant temperature chamber when placed in Ringer's cold solution.

The ring-neck pheasants were inoculated by placing the larvae down their esophagi with forceps and inducing swallowing by dropping water with a pipet down their throat. The other bird hosts were given the larvae via a stomach tube. The stomach tube consisted of a small plastic syringe with soft tygon tubing attached to it. The coiled larvae were placed in the tubing with soft brushes and water was taken up into the tubing. The tubing was then carefully placed down the esophagus of a bird and the worms expelled at about the level of the stomach. Their cages were checked periodically after infection for regurgitated worms. Mallards the summer of 1972 were inoculated as above with a stomach tube, but their bills were closed with rubber bands for approximately 8 hours, then removed.

In preparation for autopsy, the pheasants were killed by suffocation. The other birds were killed with chloroform. After death the birds were wetted down with soap and water and waste alcohol. The feathers were parted in the mid section allowing a shallow incision to be cut from throat to anus. The skin was peeled back and the external musculature searched for signs of larval migrations. After cutting through breast muscle and abdominal mesentery the internal organs were removed and separated. Close visual as well as microscopical inspection of each of the following organs was conducted: esophagus, proventriculus, gizzard, intestine, liver, and lungs. After removal of the internal organs, the abdominal wall and musculature were searched for signs of larval migration or tissue damage.

The organs were placed in Ringer's warm solution and gently teased and scraped under a dissecting binocular microscope for signs of *Eustrongylides* infection.

Eustrongylides tubifex recovered burrowing relatively free through the abdominal cavity and viscera were pulled out and placed in Ringer's warm solution. If the worms were encountered within thick fibrous tubes of connective tissue in the proventriculus, the entire proventriculus was placed in Ringer's cold solution for the slow, difficult procedure of extracting the worms. Only the mature females of *E. tubifex* could be extracted without fragmentation. Using sharp needle points, razor blades, and scissors the fibrous wall of the tubercles surrounding the encapsulated worm was slit without puncturing the worm. After a time the adult females could be carefully and slowly pulled out. In order to extract less mature females and the smaller males from the thick fibrous encapsulations the above method was tried but fragmentation always occurred. Attempts were made to pull the free ends of the worm dangling in the lumen of the proventriculus, but this also often resulted in fragmentation of the worms. Through persistant effort the fragmented worms could eventually be recovered. All worms recovered were placed in Ringer's warm solution and relaxed by gradual heating of the solution. The worms were fixed in AFA for 24 hous and stored in 70% alcohol for later study. Dehydration in glycerin alcohol was carried out as described for the larval *E. tubifex*. The larger gravid females were studied under a binocular scope while alive as well as later in a fixed cleared condition. All worms, both from fish and birds, were

studied from glycerine mounts under a compound microscope with both phase and light microscopy and from photomicrographs. Measurements were taken in mm. Scaled line drawings were produced with the aid of a Wild Microscope with drawing tubes.

In order to remedy taxonomic difficulties specimens were obtained from the U. S. National Museum Helminthological Collection in Beltsville, Maryland. These nematodes were studed and compared with the specimens of *Eustrongyldies* recovered from the experimental mallard infections. Those specimens studied were designated as *E. ignotus* and *E. tubifex*. Two males USNM 38996 were registered from an eastern green heron from Washington, D. C. as *E. ignotus*. Also registered as *E. ignotus* were one male and one female USNM 6151 from a Virginia *Ardea herodias*. Registered as *E. tubifex* USNM 37270 were three males and one female from a white-winged scoter, *Melanitla fusca deglandi* from Alaska.

In cooperation with the Wildlife Unit of the Department of Zoology at The Ohio State University, frozen proventriculi of three species of quatic birds collected from Winous Point and West Sister Island were obtained. The collection period for the birds ranged between June 27 to August 24, 1972. The following birds were shot and autopsied: 6 adult and 2 nestling *Nycticorax n. hoactli*; 4 adult, 1 morbid adult, and 7 nestling *Ardea herodias;* and 5 adult and 2 nestling *Casmerodius albus egretta*. Only one species, *N. n. hoactli*, was found to harbor an *Eustrongylides* infection.

VI. FINDINGS

Experimental Transmission Cycle Studies: Larval *Eustrongylides tubifex* taken from *Perca flavescens* were utilized in this study in experimental infections of various bird hosts. Although several previous attempts at infecting birds with larval *Eustrongylides* sp. have been undertaken in North America, none had been successful in obtaining adult forms until this study. (El Sea, 1954; Van Brand and Cullinan, 1943). In Eurasia, however, successful investigations have yielded adult *Eustrongylides excisus* from experimental infections of cormorants (Iksanov, 1958; Ciurea, 1924 in Karmanova, 1968; Dubinin, 1949; and Karmanova, 1968).

The summer of 1971, eleven of fifteen (73.3%) *Anas platyrhynchos* were successfully experimentally infected with fourth-stage larval *Eustrongylides tubifex* from *Perca flavescens*. Table 1 summarizes the results. A total of 25 of 71 worms inoculated (35.2%) were recovered. Table 2 summarizes the results of experimental mallard infections the summer of 1972. Eight of fourteen (57.1%) *Anas platyrhynchos* were successfully infected with a total of 14 of 94 worms (14.9%) recovered. After studying the adult forms recovered and comparing them with specimens of *E. tubifex* and *E. ignotus* from the U. S. Helminthological Collection the species was elucidated to be *Eustrongylides tubifex;* thus increasing the distribution of this species to the U.S.A. in addition to Western Europe and the USSR.

The adult nematodes are localized in coiled, tubular connective tissue encapsulations enmeshed through the glands of the proventriculus. A few degenerating worms were found in other visceral organs (pancreas, liver). Apparently a few larvae burrowed completely into the abdominal

TABLE 1

MALLARD EXPERIMENTAL INFECTION DATA WITH *E. TUBIFEX* FROM *PERCA FLAVESCENS* 1971

AGE AT (WKS) INFECTION	DATE INFECTED	LARVAL DOSAGE	DAYS P. I. TO AUTOPSY	# *E. TUBIFEX* RECOVERED	PROVENTRICULUS	ESOPHAGUS
4.5	July 15	4	35	1 (degen.)	X	
4.5	July 15	4	2	1 male (L-4)		X
4.5	July 15	4	7	----------		
4.5	July 15	4	9	2 male, 1 female	X	
4.5	July 15	4	14	2 female	X	
4.5	July 15	4	21	1 (degen.)	X	
4.5	July 15	4	41	----------		
6.5	July 29	3	22	3 (degen.)	X	
6.5	July 29	4	26	----------		
7.0	Aug. 3	6	7	1 male, 2 female	X	
7.0	Aug. 3	6	9	1 male, 1 female	X	
7.0	Aug. 3	6	14	----------		
7.0	Aug. 3	6	16	2 female, 2 male (degen.)	X	
7.0	Aug. 3	6	18	1 female, 1 male (degen.)	X	
7.0	Aug. 3	6	20	1 female, 2 male (degen.)	X	

TABLE 2

MALLARD EXPERIMENTAL INFECTION DATA WITH *E. TUBIFEX* FROM *PERCA FLAVESCENS*, 1972

AGE AT INFECTION (WKS)	DATE INFECTED	LARVAL DOSAGE	DAYS P. I. TO AUTOPSY	# *E. TUBIFEX* RECOVERED	PROVENTRICULUS	LIVER	PANCREAS
3	June 27	2 male, 4 female	22	2 female	X		
3	June 27	2 male, 4 female	49	3 (degen.)	X	X	
4	July 6	2 male, 4 female	25	----------			
4	July 6	2 male, 4 female	33	----------			
4	July 6	2 male, 4 female	33	----------			
4	July 6	2 male, 4 female	40	1 (degen.)			X
7	July 28	1 male, 3 female	21	----------			
7	July 28	1 male, 3 female	24	1 female	X		
9	Aug. 14	4 male, 6 female	12	----------			
9	Aug. 14	4 male, 6 female	17	----------			
9.5	Aug. 16	4 male, 6 female	15	1 female, 2 (degne.)	X		
9.5	Aug. 17	4 male, 6 female	15	1 female, 1 (degen.)	X		
11	Aug. 29	3 male, 3 female	1	1 female (L-4)	X		
11	Aug. 31	4 female	1.25	1 female (L-4)	X		

cavity, but were quickly killed by the host; while the majority of worms survived the host's digestive processes and remained in the proventricular region. Specifically they are located on the serosal side of the proventriculus within the encapulated tubes, their anterior and posterior ends protruding free from the tubes into the lumen of the proventriculus and advancing and retreating into the encapsulation with stimulation.

Male *E. tubifex* underwent their last molt 7-9 days *post infectionem*. After 16 days all males recovered were in a state of degeneration. Females molted 7-16 days *post infectionem*. Fertilized eggs were voided by gravid females 16-24 days *post infectionem*. The thick-shelled oval eggs, voided with the feces, were in the one cell stage. Polar thinnings and numerous depressions were noted on the shell surface.

Table 3 summarizes the results of experimental infections in the ring-neck pheasant, black-crown night heron, and the herring gull. Four young herring gull chicks inoculated with *Eustrongylides* larvae all proved negative to infection at autopsy. A larger sample of experimental gull hosts would have made the results more meaningful. However, in parasitology calsses held at Stone Laboratory over the past years, close to 100 gulls autopsied have shown no signs of *Eustrongylides* infection.

One nestling black-crowned night heron, inoculated with four fourth-stage *Eustrongylides* larvae died 42 hours *post infectionem* of verminous peritonitis. Before infection the young heron had exhibited a voracious appetite, but 32 hours *post infectionem* the heron was visibly shaking and refused to eat. At 36 hours *post infectionem* the heron, much weakened, failed to hold its head up. Hyperventilation and convulsions preceded

TABLE 3
RING-NECK PHEASNAT, BLACK-CROWN NIGHT HERON, AND HERRING GULL EXPERIMENTAL INFECTION DATA WITH *E. TUBIFEX* FROM *PERCA FLAVESCENS*, 1971

BIRD	AGE AT INFECTION (WKS)	DATE INFECTED	LARVAL DOSAGE	TIME P. I. TO AUTOPSY	# *E. TUBIFEX* RECOVERED	esophagus	proventriculus	muscles	liver, gizzard	intestine, mesenteries
Pheasant	3	June 23	5	12 hrs.*	4 female, 1 male (L-4)		X	X	X	X
Pheasant	3	June 23	2	2.7 days	----------					
Pheasant	3	June 23	5	2.9 days	1 male, 1 female (L-4) 3 (degen.)		X		X	
Pheasant	3	June 23	2	10 days	----------					
Pheasant	3	June 23	2	13 days	1 (degen.)				X	
Night Heron	1.5	July 13	4	42 hrs.*	1 (degen.) 1 female, 1 male (L-4)	X	X			
Gull	1-2	July 13	4	7 days	----------					
Gull	1-2	July 13	4	7 days	----------					
Gull	1-2	July 13	4	18 days	----------					
Gull	2	July 13	4	23 days	----------					

* death

death at 42 hours *post infectionem*. Upon autopsy three *E. tubifex* sub-adults (fourth-stage larvae) were recovered burrowing through the viscera. Hemorrhage was evident in the lower abdomen. None of the larvae were encapsulated. One female fourth-stage larva was encountered burrowing through the proventriculus. Another male fourth-stage larva was found burrowing from the lower esophagus into the proventriculus. A third degenerating larval *E. tubifex* was recovered within the glands of the proventriculus.

Five young ring-neck pheasants were inoculated also with larval *Eustrongylides tubifex* to ascertain the behavior of the larvae in an obvious abnormal bird host. One pheasant, given five larvae, died of verminous peritonitis 12 hours *post infectionem*. Symptoms similar to those observed in the heron were noted in the pheasant preceding death. These included hyperventilation, convulsions, wincing, and closing of the eyes. Upon autopsy all five larvae were recovered. One female fourth-stage larva was encountered burrowing into the pectoral muscles causing hemorrhage and tissue damage. A second female fourth-stage larva was recovered burrowing into the neck muscles. A third female larva was noted exiting the proventriculus into the liver. The proventricular tissue appeared quite damaged, hemorrhaging evident. The fourth female larva was encountered penetrating the mesenteries and liver. The fifth larva, a fourth-stage male, was burrowing into the wall of the lower intestine. A large blood clot was localized in the lower abdominal cavity. Two other pheasants were also positive to larval infection but the symptoms did not prove fatal as for the other pheasant. One of these pheasants upon autopsy 68 hours *post infectionem*, exhibited tissue damage of the

proventricular wall and gizzard. Though all five worms inoculated were recovered, they were inactive. Four of the larvae were degenerating. One larva was teased out of the proventricular wall, two from the liver, and two from the gizzard wall. The other pheasant, autopsied 13 days *post infectionem*, had one degenerating worm ensheathed in a host encapsulation between the proventriculus and liver. Obviously larval migrations through the pheasant, an abnormal bird host, and the nestling night heron can be quite detrimental to them.

E. tubifex developed to maturity with egg deposition by gravid females in mallards only, where no severe pathology was noted. However, in infections with high intensities the severity of proventricular malfunction would certainly increase.

Description of Larval *Eustrongylides tubifex*: Larval *Eustrongylides tubifex* were recovered from 1056 *Perca flavescens* autopsied for the population phase of this study in the Western Basin of Lake Erie. Upon investigation it was found that the larvae for descriptive purposes could be separated into two distinct morphological groups. Karmanova (1965) in experimental studies of the life cycle of *E. excisus* on the Volga delta of the USSR had infected fish with third-stage larvae from freshwater oligochaetes. In 1968, Karmanova claimed a molt to the fourth-stage occurred in larvae between 30.10-31.69 mm. long within thin encapsulations or in the free condition in the body cavity of the fish. She (1968) asserted that early fourth-stage larvae either migrated to the stomach musculature or remained in the body cavity in flat spirals within encapsulations of host fish tissue. Third-stage larval *E. excisus*,

7.99-30.00 mm long, were described by Karmanova (1968) from Volga delta fish *Neogobuis* and *Rutilus*. Though fourth-stage larval *T. excisus* were reported by Karmanova from the fish, they were not, however described.

In view of the findings of Karmanova (1965, 1968), the two larval groups of *E. tubifex* recovered from *Perca flavescens* in Lake Erie are arbitrarily considered to be third and fourth stage larvae though no molt was observed between the stages. The molting process appears complex and requires further study. Whereas the larvae designated third-stage have one cuticle, the larvae designated fourth-stage gradually acquire a double cuticle toward the end of their size range, the characters of the adult stage seen within the last cuticle. The two larval groups of *E. tubifex* from yellow perch are here described as third-stage and fourth-stage larvae.

Description: Third stage larval *E. tubifex* are localized in the body cavity of yellow perch and freshwater drum either lying free in the mesentery usually near the intestine or coiled up in a delicate thin walled capsule approximately 1.5-3.0 mm in diameter attached to the outside wall of the intestine. The larvae range in color from white to pale pink. All measurements were taken in mm.

Third-Stage Larvae (Figures 1,2,4,5) (Based on 17 specimens): Length 9.18-32.19mm (average 17.20mm), maximum width 0.120-0.426 (0.216). Width at level of outer circle of papillae 0.048-0.015 (0.062). Width at posterior end 0.078-0.237 (0.115). Twelve head papillae arranged in two circles of six each, two lateral and four submedian. Papillae digitiform. Outer circle papillae 0.006-0.015 (0.009) wide by 0.006-0.009

(0.007) long. Inner circle papillae 0.003-0.006 (0.004) wide and 0.003-0.009 (0.006) long. Nerve ring 0.081-0.120 (0.104) from anterior end. Mouth cavity 0.054-0.096 (0.079) long. Two rows of lateral papillae prominent at anterior and posterior ends, not always encountered in middle or worm. Esophagus 3.36-8.70 (5.32) long. Junction of esophagus with intestine by three lipped cardial valve. Mesenteric connectives join body wall to intestine and rectum. Anterior of rectum to posterior end 0.195-0.525 (0.297) long. Single terminal opening in both sexes. One layer of cuticle. Sexual dimorphism present. Female tail symmetrical. Male tail asymmetrical, curved ventrally. Genital tube single. Female genital tube, in form of loop, arises and terminates at posterior end. Rudiments of male genital tube ventral, extends anteriorly. Spicule sheath dorsal. Spicule not yet formed. Recutm, genital tube, and spicule sheath empty into short cuticular cloaca.

Description: Fourth stage larval *E. tubifex* are usually localized in the body cavity of yellow perch within yellowish brown granular mesentery capsules ranging in diameter from 4-15 mm (average 7 mm). Usually 1-2 larvae are found lying coiled in a flat spiral within these capsules. Up to 7 larvae were found in a single huge granular encapsulation 30 mm in diameter. Larvae on occasion are encountered burrowing into the body musculature or through the abdominal viscera. The number of larvae encountered burrowing increases as the time since death of the fish to time of autopsy increases. The larvae range in color from dark pink to dark red. Occasional aberrant white larvae are encountered. All measurements in mm.

Fourth Stage Larvae (Figures 3, 6-9) (38 speciemns): Length 32.50-93.37 mm (average 63.45 mm), maximum width 0.337-0.668 (0.502). Width at level of outer circle of papillae 0.098-0.154 (0.123). Width at posterior end 0.147-0.334 (0.290). Mouth cavity 0.082-0,196 (0.136) long. Nerve ring 0.131-0.214 (0.176) from anterior end. Head papillae in two circles of six each, two lateral and four submedian. Outer circle head papillae 0.008-0.023 (0.018) wide by 0.005-0.020 (0.013) long. Inner circle papillae 0.005-0.016 (0.010) wide by 0.008-0.016 (0.011) long. Two rows of lateral papillae on anterior and posterior ends prominent, not always encountered in middle of worm. Esophagus 7.02-26.01 (15.99) long. Grandular cells of esophagus notably large near junction of esophagus with intestine. Cardial valve three lipped. Mesenteric connectives attach intestine and rectum to body wall. Anterior of rectum to posterior end 0.804-0.891 (0.848) long in male larva. Anterior of rectum to posterior end 0.871-0.988 (0.935) long in female larva. Sexual dimorphism present. Female tail symmetrical, truncated. Male tail asymmetrical, curved ventrally. Beginnings of cup shaped muscular bursa with cuticular fringed border and internal genital "cone" evident within outer cuticle. Both sexes of fourth-stage larvae with double cuticle, especially evident at anterior and posterior ends. Genital tube single. Female genital tube arises from attachment to rectum wall, extends anteriorly, loops, and eventually gives rise to a posteriorly directed thick walled vagina, 5.34-10.68 (6.71) long. Vagina terminates with vulva at beginning of

EXPLANATION OF PLATES

Figure 1. Third-stage larva, anterior end, ventral view, showing nerve ring, head papillae, and lateral papillae.

Figure 2. Third-stage larva female, posterior end, lateral view, showing looped genital tube, rectum, and mesenteric connectives attaching intestine and rectum to body wall.

Figure 3. Fourth-stage larva, en face view, showing head papillae and mouth aperture. (en face oriented with a lateral papilla uppermost).

Figure 4. Third-stage larva, lateral view, showing junction of esophagus and intestine with three lipped cardial valve, and beginning of mesenteric connectives.

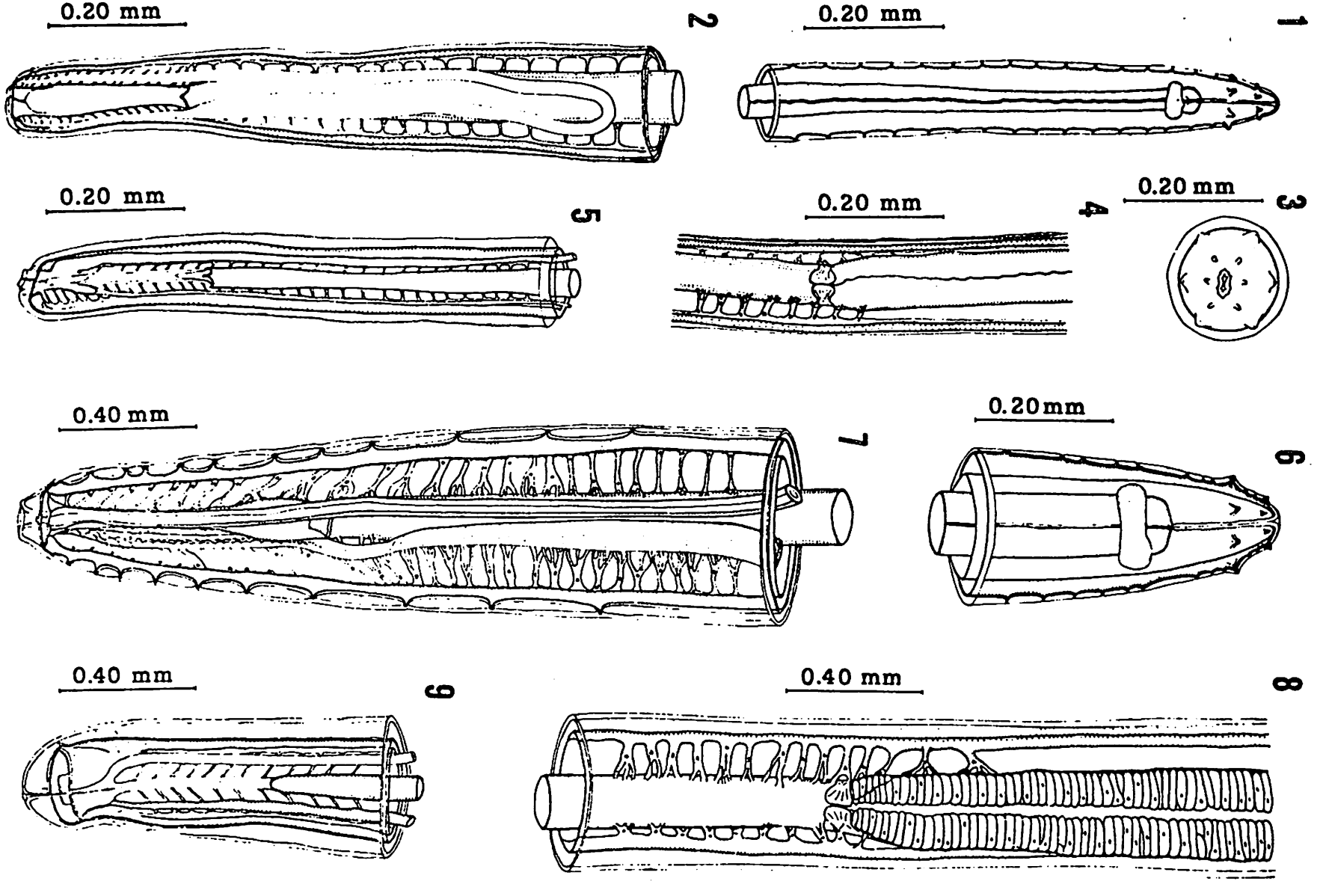
Figure 5. Third-stage larva male, posterior end, lateral view, showing rudiments of dorsal spicule sheath and ventral genital tube emptying with rectum into cloaca.

Figure 6. Fourth-stage larva, anterior end, ventral view, showing lateral papillae extending anterior to outer circle lateral head papillae.

Figure 7. Late fourth-stage larva female, posterior end, ventral view, showing double cuticle, lateral papillae, mesenteric connectives, ventral vagina with terminal vulva, and cuticular posterior extension.

Figure 8. Fourth-stage larva, lateral view, showing junction of esophagus and intestine by three lipped cardial valve, and glandular cells of posterior esophagus.

Figure 9. Late fourth-stage larva male, posterior end, lateral view, showing mesenteric connectives to intestine and rectum, dorsal spicule sheath, ventral genital tube, forming bursa with cuticular fringed border, and double cuticle.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cuticular extension of molting cuticle at posterior end. Male genital tube arises blindly in posterior half of worm, convolutes, ends in ventral tube emptying into cloaca. Muscular-like genital "cone" noted deep in the bursal cup. Occasional papillae encountered on inner surface of bursa.

Description of Adult Male and Female *Eustrongylides tubifex* (Nitzsch, 1819) Jagerskiold, 1909. *Eustrongylides tubifex* (Nitzsch, 1819) Jagerskiold, 1909. Jagerskiold (1909) in his monograph described the new genus *Eustrongylides*. Utilizing five females and one male from the Wein Museum of Natural History taken from *Colymbus septentrionalis (=Gavia stelata)*, the red-throated loon, and one male specimen from his personal Finland collection taken from *C. arcticus (=G. arctica)*, Jagerskiold redescribed *Strongylus tubifex* Nitsch, 1819 and placed it in the new genus *Eustrongylides*. *Eustrongylides tubifex* had appeared under various generic names. Jagerskiold in studying material from various museums created partial synominies of different species and considered some of the past descriptions to be actually assembly species in their composition.

The adult forms of *Eustrongylides* recovered from my experimental infections of mallards, *Anas platyrhynchos*, were compared with the U. S. Museum Helminthological Collection specimens of *E. tubifex* and *E. ignotus* and also with the descriptions of the different species of *Eustrongylides* as written by Jagerskiold (1909) and discussed by Karmanova (1968). The species of nematode found in the experimental mallard infections was established to be *E. tubifex* (Nitzsch, 1819) Jagerskiold, 1909. Key characteristics used to elucidate the species were: (1) 12 papillae about the mouth, (2) the lack of a deep cleft in the male bursa, (3) the larger

size of the outer circle papillae, (4) the fringed cuticular border of the male bursa, and (5) the straight spine-like structures terminating the inner circle papillae. This combination of characters distinguishes *E. tubifex* from the other species of the genus *Eustrongylides*.

In as much as the adult *E. tubifex* recovered from the experimental mallard infections were much larger in size, males 56.62-86.64 mm long and females 65.00-120.00 mm long, than those of Jagerskiold (1909), male 34.0 mm long and females 35.0-44.0 mm long, a description of the adult male and female *E. tubifex* taken from *Anas platyrhynchos* appears desirable.

Description: Diotophymatida, Dioctophymatina, Dioctophymatoidea, Dioctophymatidae, Eustrongylidinae. Nematodes of large size. Body spindle shaped, gradually widening to middle. Narrower anterior and posterior ends hang free into lumen of proventriculus. Thick middle part of worm located within connective tissue tubular capsules on side of serosal layer of proventriculus. Live nematodes a rose-beige color. Cuticle of anterior and posterior end thicker than cuticle of miccle part of worm. Transverse striations of cuticle most prominent on anterior and posterior ends, that covering middle part of body appears smooth.

Mouth opening, lacking lips, of variable form from triangular to hexagonal to round depending on condition of nematode. Head with 12 papillae arranged in two concentric circles. Each circle consists of 6 papillae, two lateral and four submedian. Papillae of inner circle much smaller than huge papillae of outer circle. Each papilla of inner circle provided with straight spine-like structure at its summit. This spine

comparatively long, with straight lateral walls in shape of cone. Each prominent papilla of outer circle, having wider base as well as greater height, provided at summit with button-like formation.

Two rows of lateral papillae arranged lengthwise along body of nematode, especially prominent on anterior and posterior ends of body. At head end lateral papillae often begin just behind laterally positioned papillae of outer circle of head papillae, but sometimes before.

Large nerve rings located near anterior end, just behind start of esophagus. Mouth capsule, cuticular and narrow, opens into esophagus. Esophagael lumen triangular in section. Esophagus widens posteriorly somewhat, sometimes anterior portion of esophagus winds or loops. At junction of esophagus into intestine, three lipped cardial valve. Intestine opens into cuticular rectum. Rectum terminated by anus in females. Rectum opens into cloaca in males. Numerous mesenteric connectives clearly visible adjoining body wall to intestine and rectum.

Male (Figures 10-13) (6 specimens): Length 56.62-86.84 mm (80.73), maximum width 0.97-1.07 (1.00). Esophagus 11.80-15.40 (13.59) long. Nerve ring 0.134-0.214 (0.172) from anterior end. Mouth cavity 0.066-0.134 (0.116) long. Width of body at level of outer circle of papillae 0.167-0.267 (0.200). Width of body at posterior end (bursal width) 0.400-0.467 (0.421). Outer circle head papilla width 0.042-0.050 (0.045). Outer circle papilla height 0.032-0.040 (0.036). Inner circle head papilla width 0.015-0.032 (0.025). Inner circle papilla height 0.016-0.026 (0.023) (including spine-like structure). Genital apparatus consists of genital tube, spicule with spicule sheath, and copulative bursa. Genital tube hologonic, single. Blind end

of testes arises in posterior half of worm, highly convoluted. Genital tube opens into cloaca below rectum. Spicule very long, 8.6-10.2 (9.15), thin, ending in sharp needle-like point. Spicule located in muscular sheath. Proximal end of hollow spicule wider. Muscular sheath of spicule extends forward past proximal end of spicule. Copulative muscular bursa terminates at tail end. Inner surface of bursa with very small sensitive papillae. Bursal edge in form of delicate cuticular fringe. Cone-shaped structure or genital "cone" deep within bursal cup. Rectum plus cloaca length 0.817-1.340 (1.128).

Female (Figures 10, 14) (10 specimens): Length 65.0-120.0 mm (87.9), maximum width 1.65-4.30 (2.29). Esophagus 8.0-22.5 (18.8) long. Nerve ring 0.167-0.335 (0.221) from anterior end. Mouth cavity 0.067-0.134 (0.113) long. Width of body at level of outer circle of papollae 0.200-0.267 (0.223). Width of body at posterior end 0.267-0.536 (0.394). Outer circle head papilla width 0.047-0.067 (0.054). Outer circle papilla height 0.020-0.048 (0.031). Inner circle head papilla width 0.023-0.054 (0.032). Inner circle papilla height 0.013-0.040 (0.021) (includes spine-like structure). Rectum lenfth 0.17-1.27 (0.99). Posterior end blunt, rounded. Anus terminal. Genital tube single, hologonic. Vulva positioned terminally. Muscular vagina long, 18.76-21.43 (20.10).

Eggs of oval form with polar thinnings, thick shelled, surface with numerous depressions. Dimensions 0.030-0.036 (0.038) wide by 0.050-0.063 (0.059) long.

HOST: *Anas platyrhynchos* (experimental)

SITE OF INFECTION; Proventriculus

LOCALITY: Lake Erie

Figure 10. Adult male, en face view, showing round mouth aperture and 12 head papillae arranged in 2 circles of 6 papillae each. (en face oriented with lateral papilla uppermost).

Figure 11. Adult male, lateral view, showing mesenteric connectives, dorsal spicular sheath with spicule, ventral genital tube, bursa with cuticular fringed border, and genital "cone" within bursal cup.

Figure 12. Adult male, anterior end, lateral view, showing head papillae, nerve ring, and body wall extensions to mouth cavity.

Figure 13. Late fourth-stage male molting to adult male 9 days post infectionem, lateral view, showing molted cuticle, extended spicule, muscular contraction of bursa, and looped genital tube over intestine and rectum.

Figure 14. Adult female, posterior end, ventral view, showing muscular vagina with eggs, terminal vulva ventral, and terminal anus at end of rectum.

Figure 15. Egg, voided 16 days post infectionem in one cell stage, showing thick shell with surface depressions, and polar thinnings.

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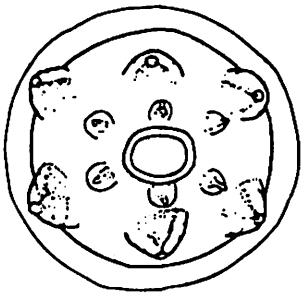

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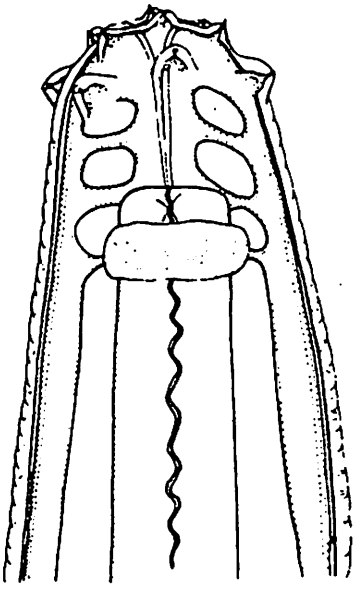

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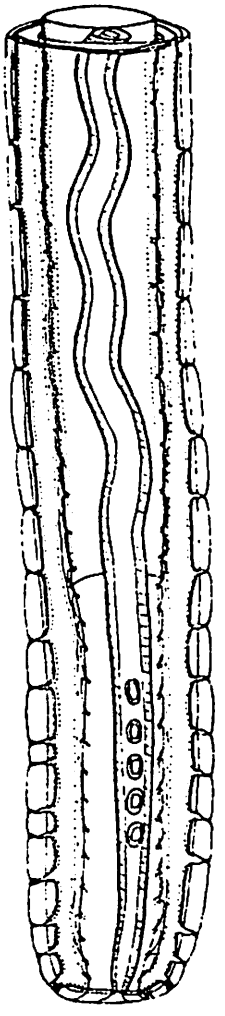

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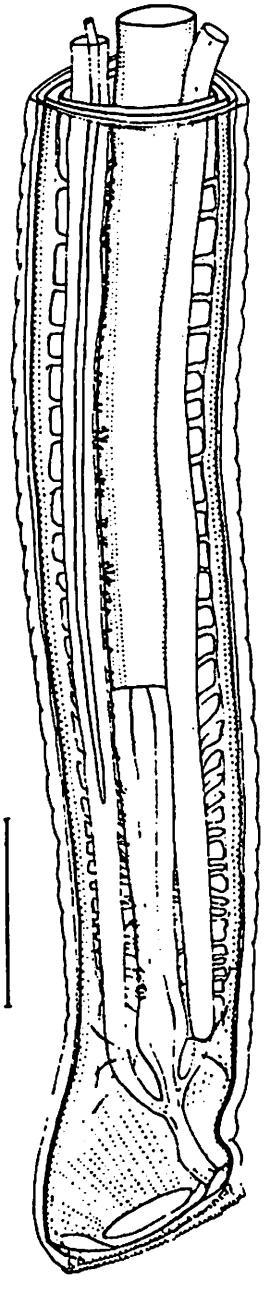

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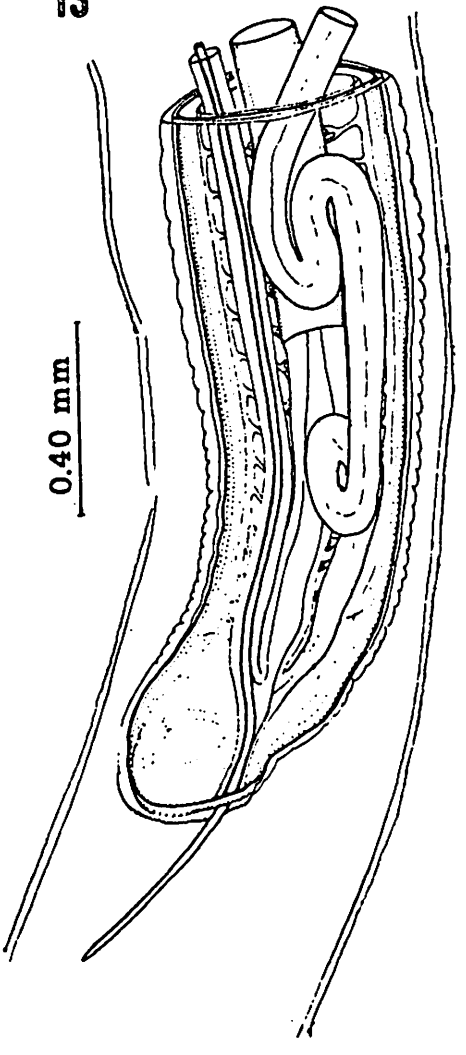

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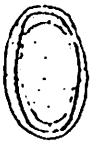

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Description of Pathology of Host Tissue: In order to ascertain the pathology to fish tissues, the granular capsules surrounding the coiled *E. tubifex* larvae in the mesentery and fatty tissue of the body cavity of the yellow perch were sectioned and stained according to Mallory's technique. The sections indicate that the larvae are encapsulated by connective tissue hyperplasia in which are trapped isolated pieces of pancreatic tissue. The pancreatic tissue nearer the periphery of the capsule appears normal, while that more deeply trapped appears abnormal and resembles liver tissue.

Presently sections of mallard proventricular tissue with encapsulated adult *Eustrongylides tubifex* entwined through the glands of the proventriculus on the serosal side are being conducted. As of yet no comment can be made on the pathology to the bird other than a gross description as previously described.

Population Studies: In order to understand the maintenance cycle of the parasitic worm *Eustrongylides tubifex* one needs to investigate the natural hosts harboring the infection and at what level the infection is expressing itself in the hosts through time. Thus far in this study we have been investigating both intermediate fish hosts and final bird hosts. Our studies of fish have been focused on yellow perch, *Perca flavescens* and to a lesser extent freshwater-drum, *Aplodinotus grunniens*. Our investigations of natural bird hosts are in a preliminary stage. We are beginning to investigate possible infections in herons in the Western Basin region. These herons include the black-crowned

night heron, *Nycticorax nycticorax hoactli;* the great blue heron, *Ardea herodias;* and the American egret, *Casmeroduis albus egretta.* Occasional ducks are autopsied when available for autopsy.

Bangham (1972) revealed data of a 1957 resurvey of Western Lake Erie and found five fish species to harbor *Eustrongylides* sp. larvae: *Perca flavescesn, Aplodinotus grunniens, Cottus bairdi, Percina caprodes semifasciata,* and *Ictalurus punctatus.* In our investigations thus far we have recovered larval *E. tubifex* from *Perca flavescens, Aplodinotus grunniens, Micropterus dolomieui,* and *Ictalursu punctatus.* Literature reports, especially those from Eurasia (Karmanova, 1968), indicate yellow perch as playing a major role in the maintenance of *Eustrongylides* larvae in fish populations. In the past, the levels of infection reported for perch from Lake Erie have not been intense. In a survey of Lake Erie fish parasites, Bangham and Hunter (1939) failed to detect any sign of *Eustrongylides* sp. from any of 79 species checked. Bangham (1972) reporting 1957 results of a summer survey of the Western Basin of Lake Erie found eight of ninety-three *Perca flavescens* infected with *Eustrongylides* sp. Also infected were two of eighty-eight *Aplodinotus grunniens,* one of fifty-eight *Percina caprodes,* two of three *Cottus bairdi,* and one of thirty-nine *Ictalurus punctatus.* Dechtiar (1972) between 1961-1969 found three of 150 *P. flavescens* infected with *Eustrongylides* sp. Vendeland (1968) in a survey of *A. grunniens* in the region of South Bass Island Lake Erie, found eighteen of thirty-three drum infected with either viable or degenerating *Eustrongylides* sp. Only five live worms were found (up to 15 mm long). Dead worms up to 30 mm long were found in

mesentery capsules while the live worms were recovered from small capsules attached to the intestinal wall.

From our investigations of freshwater-drum, *Aplodinotus grunniens*, the infection levels of larval *Eustrongylides tubifex* are much lower than those found in yellow perch, *Perca flavescens*. This coming year we will investigate more closely the levels found in the drum. Our investigations thus far indicate that the majority of live *Eustrongylides tubifex* larvae recovered are the younger third-stage larvae while a few fourth-stage (longer than 30.0 mm) larvae have been found. Also now evident is that the majority of encapsulations in the body cavity of freshwater-drum localized along the mesentery near the intestine are encapsulations of dead third-stage *Eustrongylides tubifex* larvae. Apparently most of the third-stage larval *Eustrongylides tubifex* larvae entering freshwater drum from the first intermediate host (possibly freshwater oligochaetes) do not develop further but are killed by the fish host tissue reaction resulting in many small hardened encapsulations along the mesentery near the intestine of the fish.

A rather rigorous analysis of the infection levels of larval *Eustrongylides tubifex* in yellow perch, *Perca flavescens* has been undertaken. Population data was compiled and analyzed by the computer in the form of scatter diagrams, cross tabulations, and certain statistical parameters.

Table 4 summarizes the statistics for the total yellow perch sample and also the three different age-size classes of perch. Data for establishing the age classes was obtained from Herdendorf and Hair (1972). Because of the large overlap in the size of the age classes 2 years old and older, these fish were all lumped into one age-size class,

TABLE 4

SUMMARY POPULATION DATA OF *EUSTRONGYLIDES TUBIFEX* FROM *PERCA FLAVESCENS* OVER ENTIRE COLLECTION PERIOD (June-Aug. 1971, May-Oct., 1972)

AGE SIZE CLASS OF FISH	SAMPLE SIZE OF FISH	% INF	MEAN WORM BURDEN OF INFECTION ($\bar{X}$inf)	MEAN WORM BURDEN OF POPULATION ($\bar{X}$pop)	RANGE OF WORMS	STANDARD DEVIATION OF $\bar{X}$pop
Entire sample	1056	37.0	1.879	0.696	0-15	1.352
YOY (4.4-11.9 cm)	239	10.0	1.167	0.117	0-4	0.403
I (12.0-17.5 cm)	373	41.8	1.589	0.665	0-7	1.007
II+ (17.6-34.6 cm)	444	47.8	2.175	1.034	0-15	1.765

II+. Immediately obvious is that the older, larger perch are more highly infected than younger, smaller fish. This is bourne out in each of the paramesters percentage infection, $\overline{X}$inf,$\overline{X}$pop, and range. The increase in infection levels with size of fish probably reflects both a change in feeding habits of the fish and accumulation of worms through time. Price (1963) in his study of the food habits of Lake Erie fish stated that as perch size increased their feeding on zooplankton decreased. Also indicated with fish age is an increase in more benthic feeding habits. In as much as the first intermediate host is thought to be freshwater oligochaetes, transmission of *E. tubifex* from oligochaetes to perch would have a higher probability with the older, larger perch. Another factor operating in the increased infection levels in the older perch is the increased carnivorous habits of older perch, it being possible that the larval *E. tubifex* could be transmitted from one fish to another fish. Von Brand (1944) successfully transmitted larval *E. ignotus* from one fish to another. The gradual increase with fish size in the mean number of worms of infected fish ($\overline{X}$inf), I believe, reflects an accumulation of the worms in the fish host through the life of the fish. Von Brand (1943) reported culturing *E. ignotus* for four years in the larval stage taken from *Fundulus diaphanus*. This accumulation is also shown in the wider ranges of worms in the older fish; for example, YOY= 0-4 while II+=0-15 worms.

Table 5 depicts statistics for the number of worms recovered from different tissue sites in yellow perch. The most probable locale of infection are the pink to yellowish-brown granular encapsulations in the

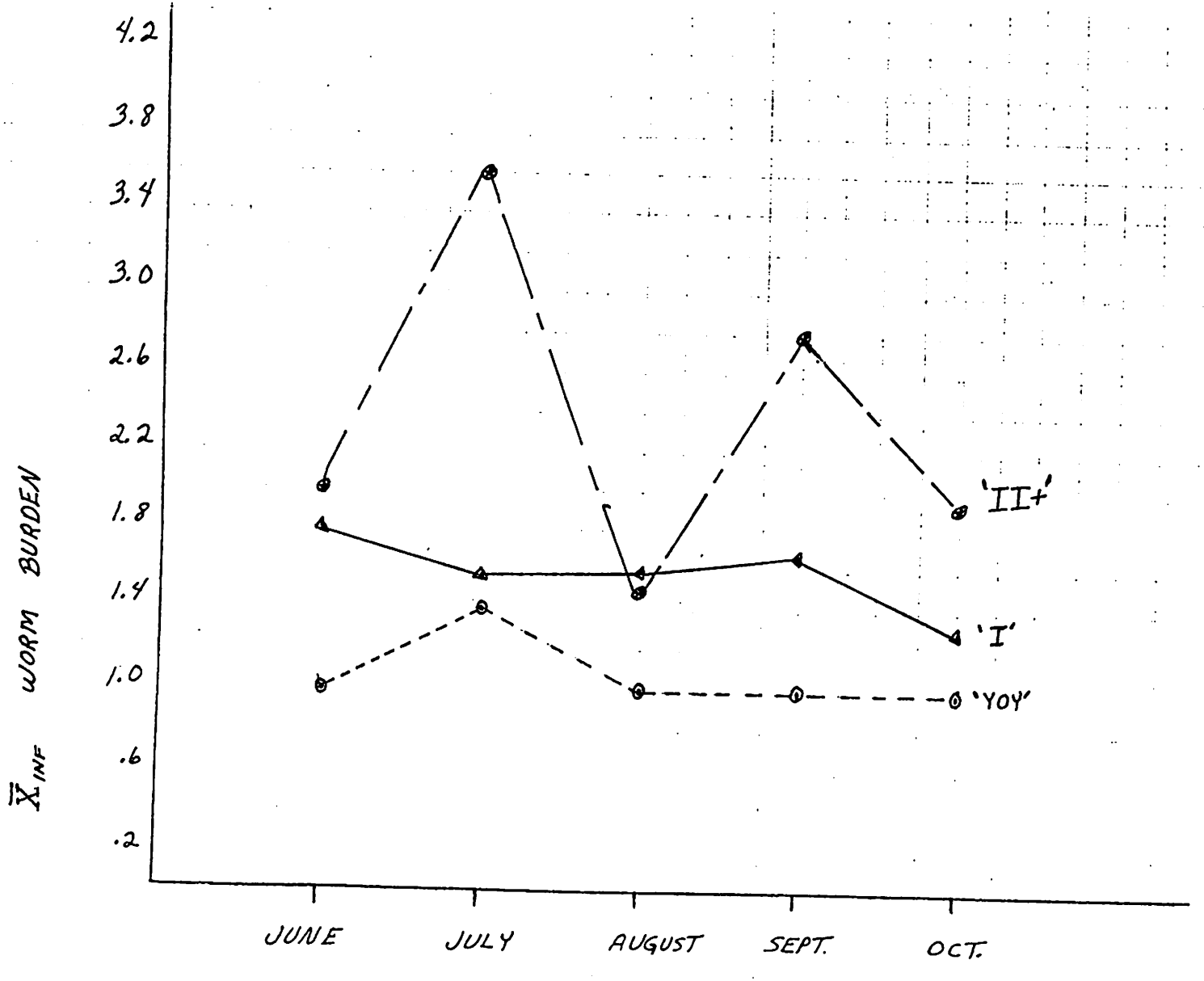
TABLE 5
SITE SELECTION BY *EUSTRONGYLIDES TUBIFEX* IN *PERCA FLAVESCENS*
FOR ENTIRE FISH SAMPLE (June-Aug. 1971, May-Oct., 1972)

TISSUE SITE	# FISH	TOTAL # *E. tubifex*	MEAN # WORMS	RANGE	S. D.
Capsules from mesentery and fatty tissue	359	662	1.844	1-14	1.571
Free in mesentery	13	13	1.000	1	-----
Burrowing (viscera, muscles)	32	46	1.438	1-4	0.759
Capsules on outer intestinal wall	3	3	1.000	1	------
Gonads	2	2	1.000	1	-------
Pepsin digest of viscera and mesentery	9	9	1.000	1	-------

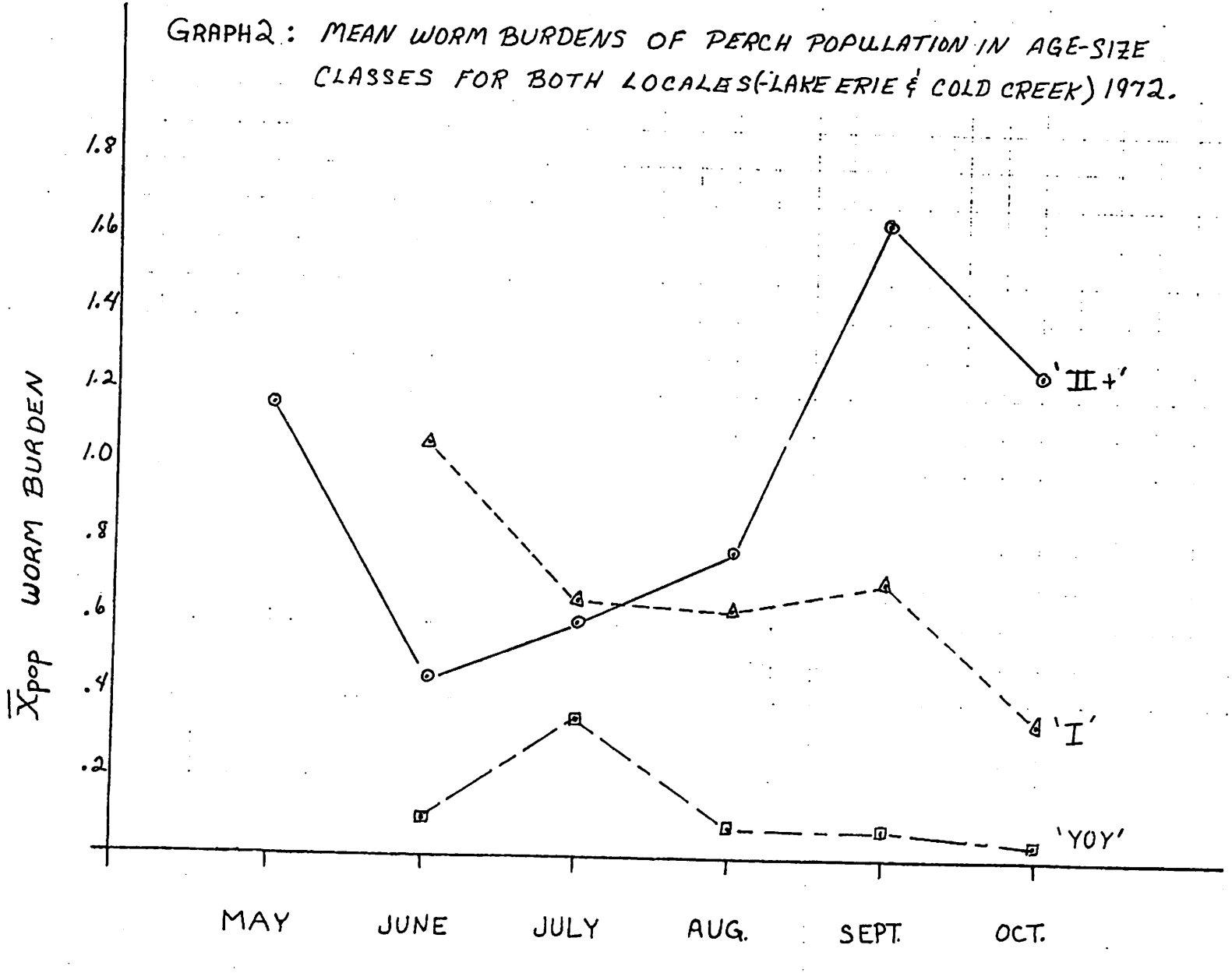
mesentery and fatty tissue. The next most frequent place of occurrence was in the musculature or viscera with the larvae actively burrowing. The granular mesentery capsules were always encountered in the body cavity from which the larvae had exited. It was observed that perch not autopsied fairly shortly after collection exhibited the greater number of actively burrowing larvae. Possibly accounting for the activation of the larvae are several factors: degeneration of the perch flesh and increase in temperature and stress to the fish when trawling them up from the lake bottom. In any case burrowing is probably related to some sort of stress factor. Fourth-stage larvae were predominately encountered in the above two sites, mesentery capsules and burrowing in the musculature and viscera. Third-stage larvae were found in either small pink capsules in the mesentery near the intestinal wall, on the outer intestinal wall, or free in the mesentery near the intestine. Very few larvae were found in or on the gonads. Some larvae were also recovered from YOY perch by using the pepsin digest technique of of the mesentery and viscera. From the results it is believed that upon ingestion of the larvae from the first intermediate host the third-stage larvae quickly exit the digestive tract into the body cavity where they gradually become encapsulated by a hyperplasia of connective tissue. A molting process not understood as of yet and considerable growth occurrs while the worm is encapsulated. With appropriate stimulation the large red fourth-stage larvae sometimes exit the capsules and burrow about in the fish viscera and musculature.

Graphs 1 through 4 illustrate the mean number of worms recovered from yellow perch in the two different locales or averaged together as affected

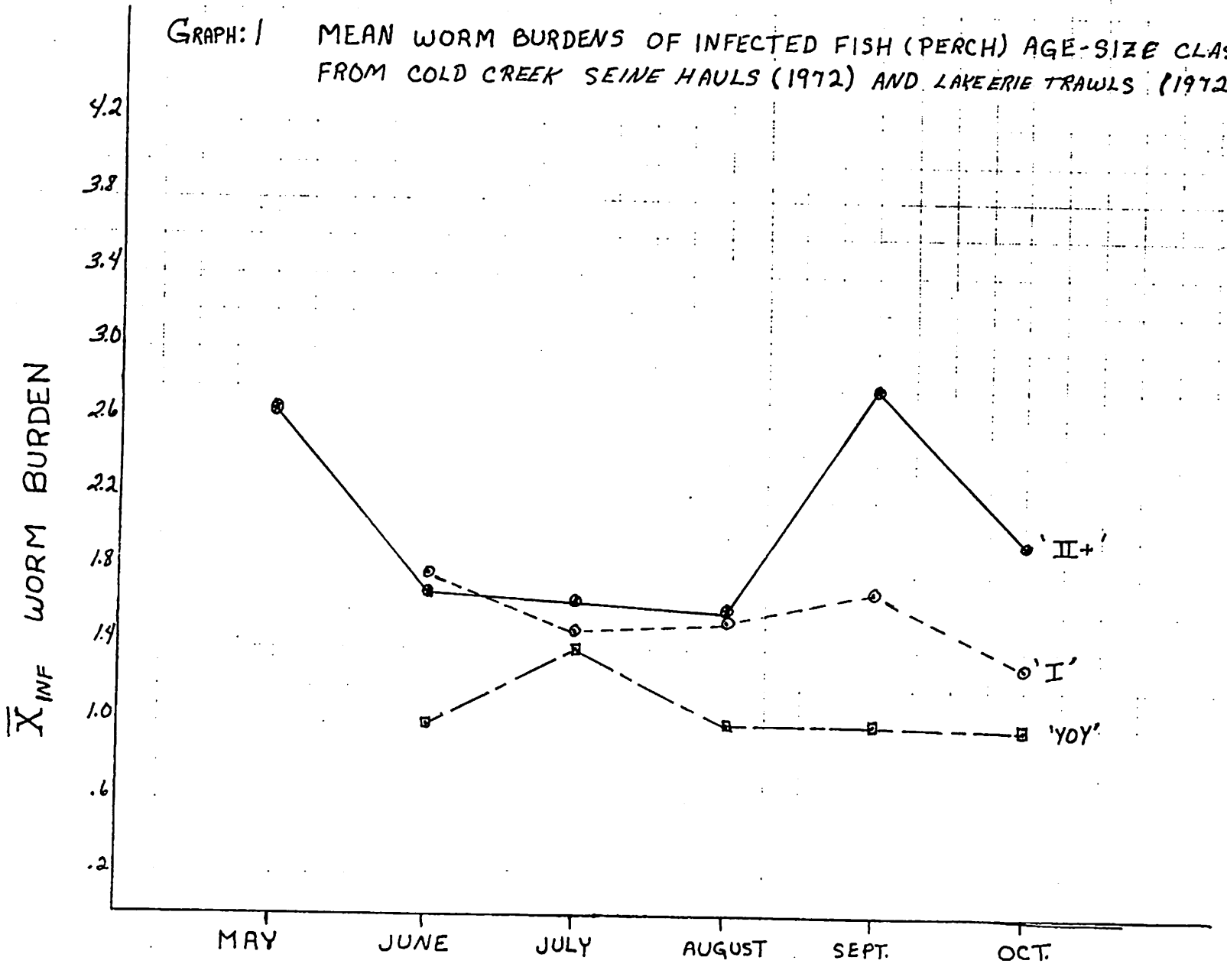
GRAPH 3 : MEAN WORM BURDENS OF INFECTED PERCH AGE-SIZE CLASSES FROM LAKE ERIE TRAWLS (1972).



GRAPH 2: MEAN WORM BURDENS OF PERCH POPULATION IN AGE-SIZE CLASSES FOR BOTH LOCALES (LAKE ERIE & COLD CREEK) 1972.

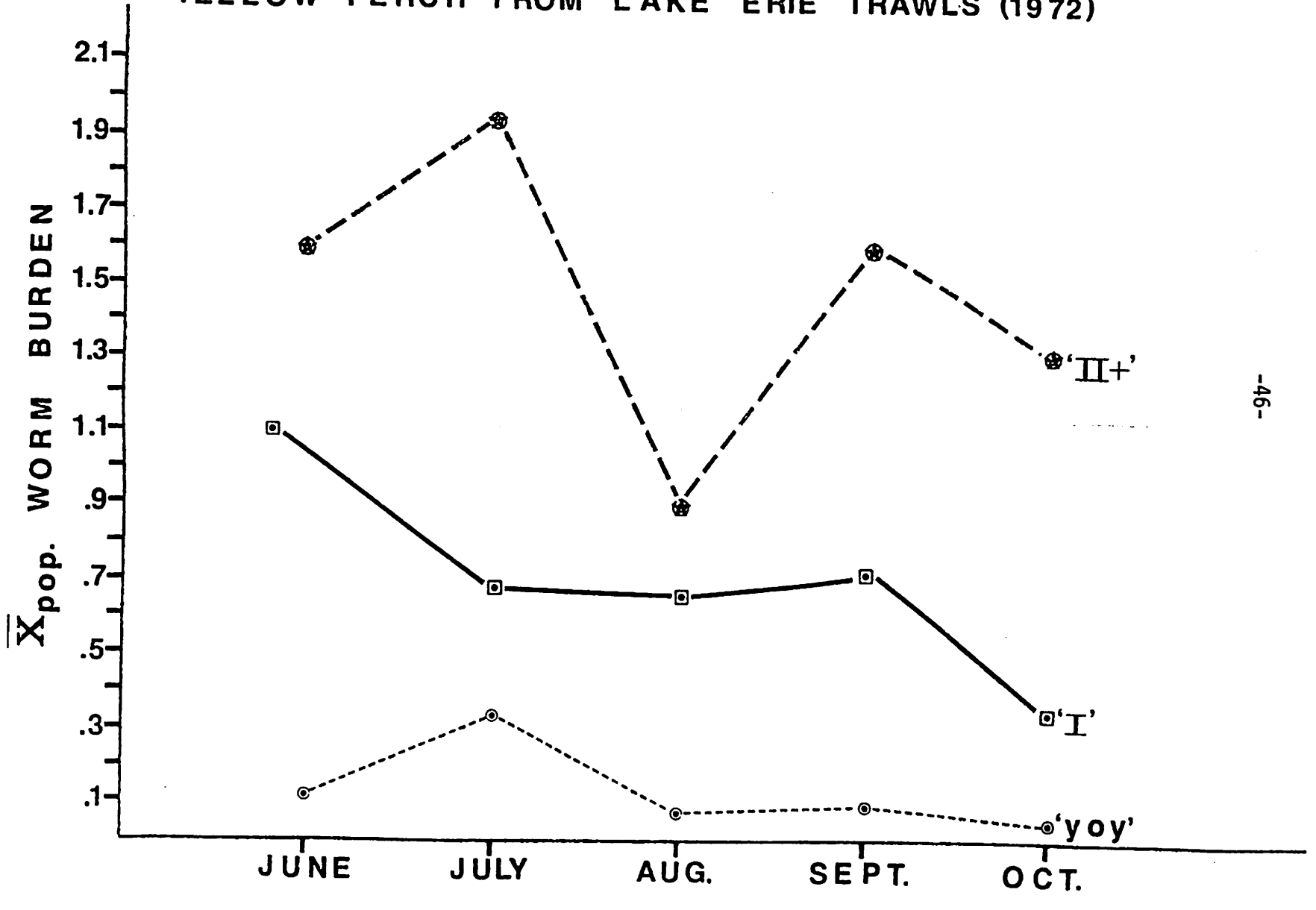


GRAPH: 1 MEAN WORM BURDENS OF INFECTED FISH (PERCH) AGE-SIZE CLASSES FROM COLD CREEK SEINE HAULS (1972) AND LAKE ERIE TRAWLS (1972).



GRAPH 4:

INFECTION LEVELS OF EUSTRONGYLIDES TUBIFEX IN YELLOW PERCH FROM LAKE ERIE TRAWLS (1972)

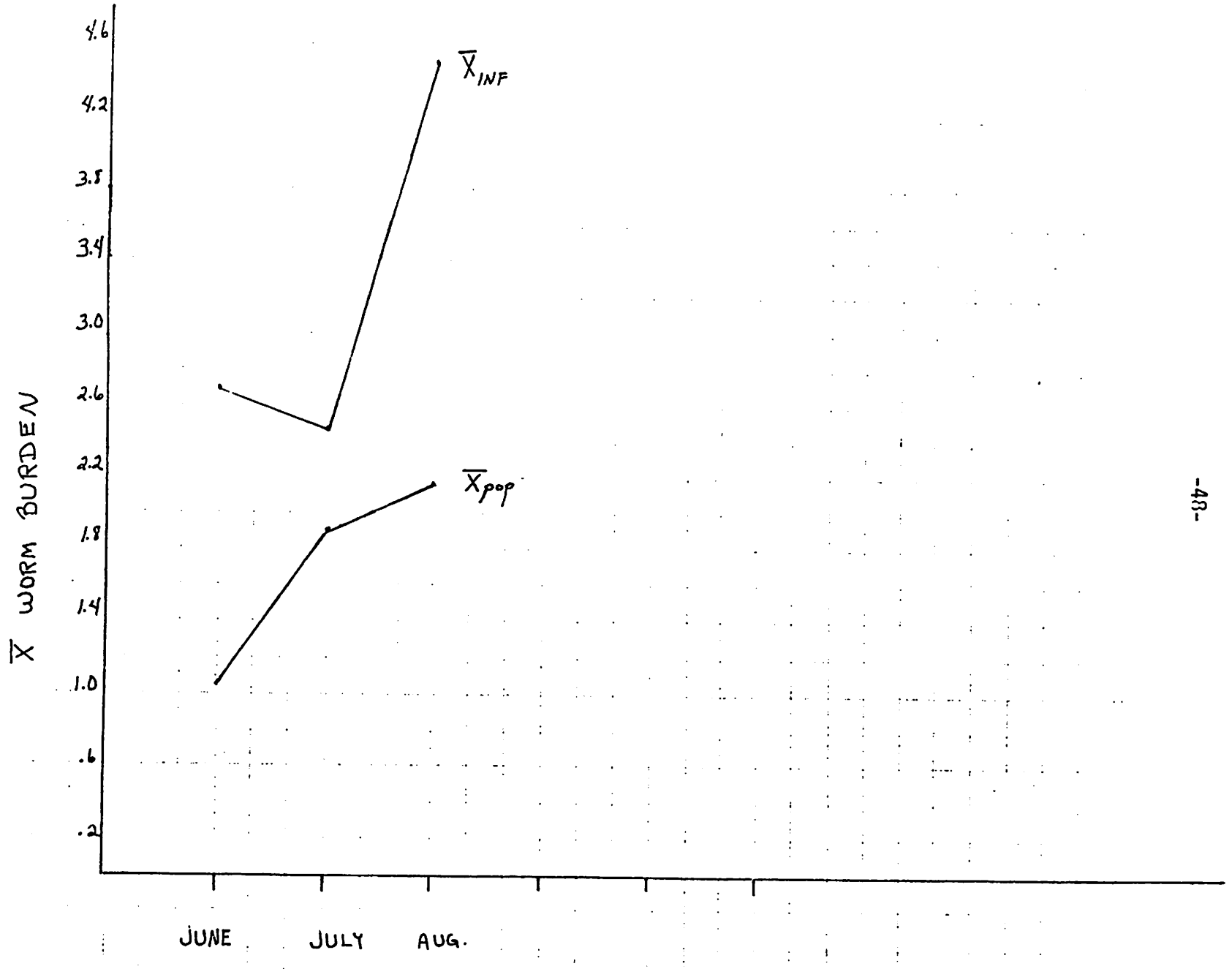


by the month sampled. In all four graphs the older fish have higher worm burdens throughout the collection period with a few small exceptions. The mean worm burdens of infected fish ($\bar{X}$inf) are closer for the three different age-size classes than those for the sample population ($\bar{X}$pop). The variance in mean number of worms over the collection period is considerable within perch age-size classes, particularly age classes I and II+. No attempt will be made to explain the oscillations as of yet on any biological basis. We hope to compare our results this coming year and then try to evaluate the worm fluctuations through time.

Graph 5 illustrates data from 1971 for the age-size class II+. In one graph one notes the mean worm burden of infected fish logically being higher than for the entire sample of that population of perch. In 1971 it appearred the mean number of worms increased over the summer. Values obtained that summer were also considerably higher than in 1972. But again large variation makes interpretation difficult.

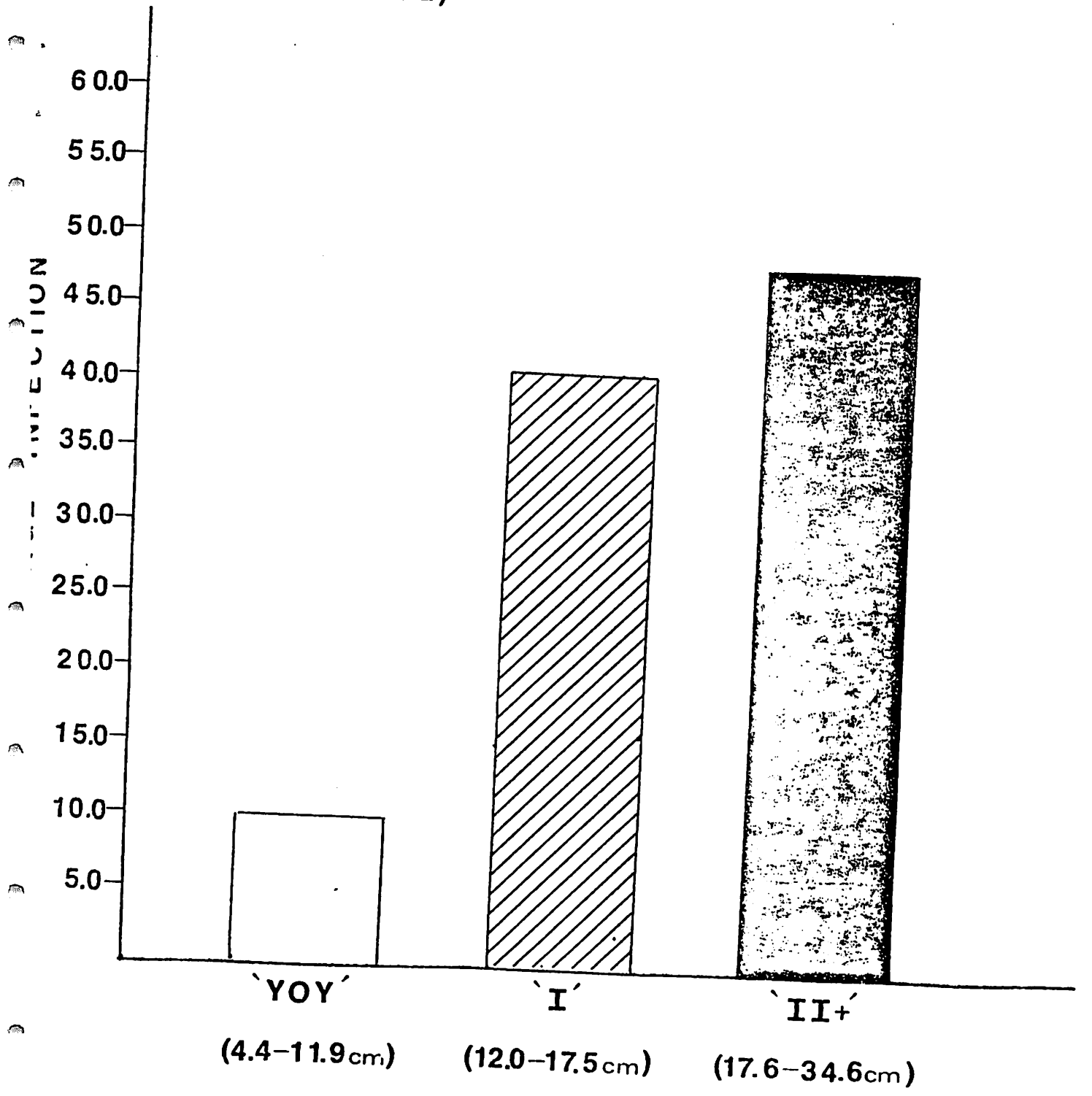
Graph 6 illustrates with a bar graph the relative abundance (%) of larval *E. tubifex* in the three different age-size classes of yellow perch. As noted earlier the older and larger the fish, the greater the probability of being infected.

Graph 7 shows the frequency distribution for all the age-size classes of yellow perch. The graph shows that small numbers of fish had many worms, whereas large numbers of fish had few or no worms. The distribution indicates a clumping in number of worms around one or two worms. One can also note the increase in level of infection the older and larger the perch.

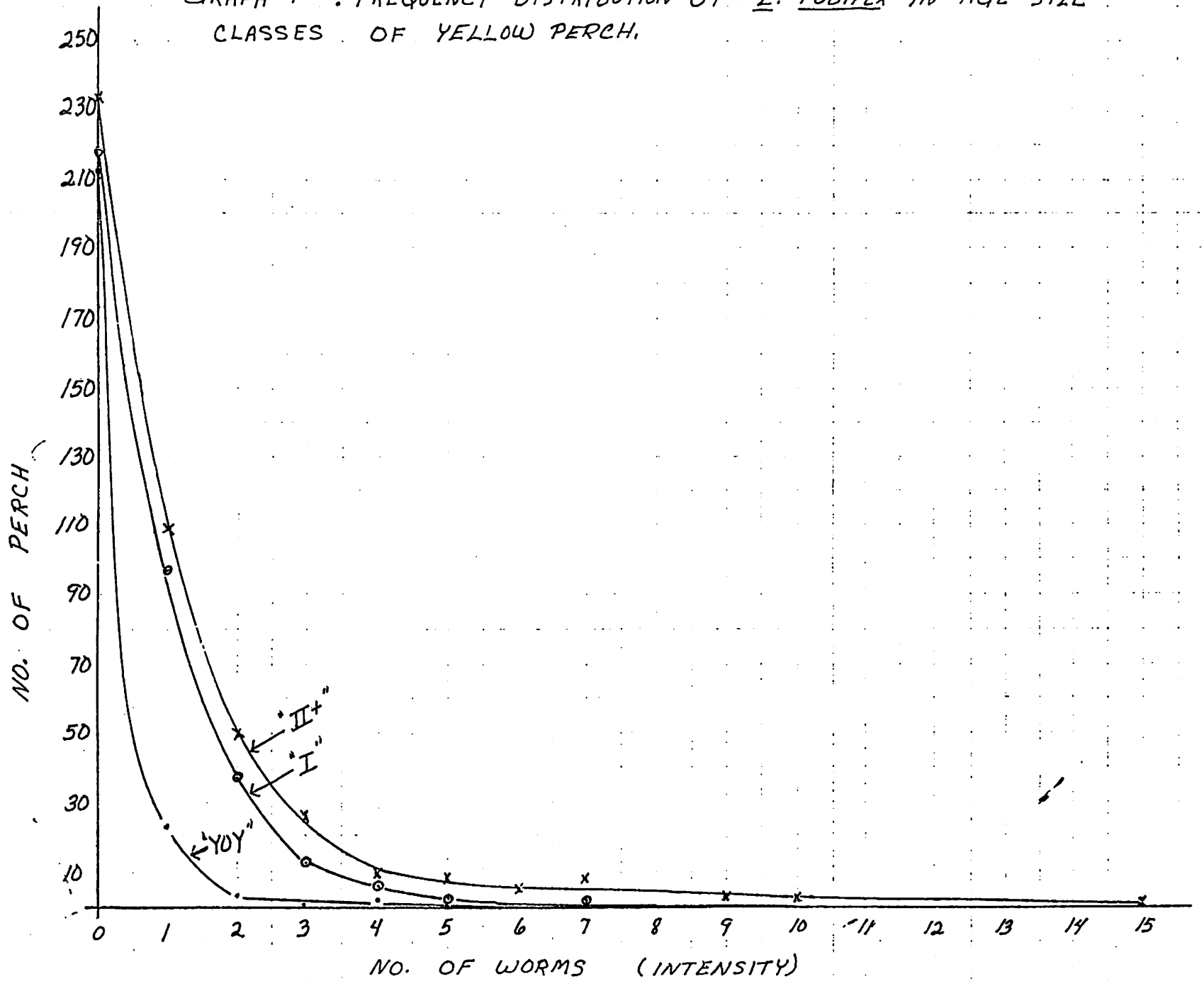


GRAPH 5 : $\overline{X}$ WORM BURDEN OF AGE-SIZE CLASS "II+" FROM COLD CREEK SEINE HAULS. (1971)

APH 6: INFECTION LEVELS OF E. TUBIFEX IN AGE-SIZE CLASSES OF YELLOW PERCH (MAY-OCT. 1971-1972)



GRAPH 7 : FREQUENCY DISTRIBUTION OF *E. TUBIFEX* IN AGE-SIZE CLASSES OF YELLOW PERCH.



From the information gathered we can conclude that yellow perch, *Perca flavescens* do indeed play a major role in maintaining *Eustrongylides tubifex* in the Lake Erie region. No definite seasonal pulse of infection in the perch population indicates that the perch are acquiring the infection throughout the collection period. Also probable is that the larvae can live in the fish beyond a year in as much as fourth-stage larvae are found from early spring to late fall. No statement concerning the third-stage larvae can safely be made as of yet since few have been recovered. Now it appears that occasionally throughout the collection period one may encounter a third-stage larvae in the perch.

Quite helful in analyzing the differences reported in relative abundance of eustrongylidosis in *Perca flavescens* in past years (Dechtiar, 1972; Bangham. 1972) was a preserved *Perca flavescens* collection sent by the Bureau of Sport Fisheries and Wildlife in Sandusky, Ohio. Table 6 summarizes the results of our autopsies of the fish.

TABLE 6
SUMMARY OF *EUSTRONGYLIDES TUBIFEX* INFECTION IN *PERCA FLAVESCENS* FROM SANDUSKY BAY AT JOHNSON ISLAND

AGE-SIZE CLASS	DATE	#FISH POSTED	% INF.	$\bar{X}$pop	$\bar{X}$inf	RANGE
I	13 June 1960	17	17.64	0.24	1.33	0-2
II+	13 June 1960	5	40.00	0.40	1.00	0-1
YOY	10 Oct. 1960	54	-----	----	-----	---

The results from 1960 are very similar to that we are obtaining in our present investigations. It appears likely that Bangham's (1972) survey of 1957 and Dechtiar"s (1972) work from 1961-1969 missed a lot of the larval *Eustrongylides tubifex* in the mesentery and fatty tissue encapsulations.

In order to ascertain the main definitive aquatic bird hosts maintaining the infection in yellow perch in Lake Erie, an investigation of local heron populations is being conducted. Frozen proventriculi and esophagi from herons of the Lake Erie region were autopsied f possible *Eustrongylides tubifex* infections. Herons investigated were the American egret, *Casmerodius albus egretta;* the great blue heron, *Ardea herodias;* and the black-crowned night heron, *Nycticorax nycticorax hoactli.* The initial collections of the herons by the Wildife Unit at The Ohio State University were made between the 27th of June and the 24th of August 1972 at West Sister Island, Lake Erie and at Winous Point. In this preliminary investigation the following numbers of herons were autopsied: 6 adult and 2 nestling black-crown night herons; 4 adult, 1 morbid adult, and 7 nestling great blue herons; and 5 adult and 2 nestling American egrets. No sign of eustrongylidosis was noted in the great blue herons or the American egrets. Two adult balck-crown night herons had badly degenerating nematodes in the of the proventriculus that probably were degenerating *Eustrongylides tubifex* larvae. Another adult black-crown night heron had two sub-adult *Eustrongylides tubifex* specimens, one male and one female. The male was badly degerated. The female worm had caused a small tubercle and some tissue damage in the glands of the proventriculus. One nestling night heron harbored 6 larval *Eustrongylides tubifex.* Two of the larvae were recovered free in the lumen of the proventriculus, close in development to those stages recovered from yellow perch. The other four larvae were in various stages of degeneration in the glands of the proventriculus. Based on this preliminary investigation black-crown night herons appear more susceptible to *Eustrongylides* infection than American egrets or great blue herons in the Lake Erie region. Samples of

more herons are being obtained from the Wildlife Unit this year. A spring collection (May 14) from West Sister Island is beginning to be autopsied. Two additional collections will be made in mid summer and fall. The samples consist of approximately 15 of each bird species totaling about 45 bird specimens for each of the three aquatic bird species to be autopsied.

Upon further investigations fish-eating ducks may prove to be the main definitive host for adult *E. tubifex*. In Eurasia loons (Karmanova, 1968) appear to play a major role in maintaining the adult worms. On July 4, 1972 an immature male red-breasted merganser, *Mergus serrator*, was found dead and emmaciated 10 feet from Fishery Bay on the shore of South Bass Island. Upon autopsy one live adult female *E. tubifex* was encountered coiled in a fibrous, tubular encapsulation in the glandsof the proventriculus. One or two degenerating worms were also contained encapsulated in the proventricular tissue. Upon inspection of the eggs that were voided by the worm, they appeared unfertilized. The characteristic shell and form of *Eustrongylides* eggs were lacking. Mergansers then definitely harbor natural infections of adult *Eustrongylides tubifex* in the Lake Erie region. The relative abundance of the worms in mergansers, though, remains to be seen.

VII. RECOMMENDATIONS

Several recommendations can be made on the basis of the past year's research in future management. The most obvious recommendation concerns stocking of ponds and lakes with fish, particularly yellow perch. Our studies indicate that the younger, shorter perch, especially young of the year, have a much lower level of infection of larval eustrongylidosis than

the older, larger fish. The relative abundance (percentage infection) of the worm in the age-size classes is as follows: YOY-10.0%; I-41.8%; and II+-47.8%. Thus stocking with young perch would reduce the chances of introducing the disease into a new locality especially if local aquatic bird populations were in residence to complete the transmission cycle. In conjunction with stocking ponds would be control of fish eating birds. Although we have not gathered enough data yet on the aquatic birds maintaining eustrongylidosis in nature, we feel safe in assuming that the definitive hosts are mainly fish-eating ducks. Thus if these birds could be kept off small bodies of water containing yellow perch, the levels of infection should drop. Logistic problems in such a control measure on Lake Erie are abvious.

Another factor needing further study concerns freshwater oligochaetes, the suspected first intermediate host. In that oligochaetes often thrive in polluted, organic waters, measures aimed at pollution abatement would reduce oligochaete abundance and logically decrease transmission of eustrongylidosis from oligochaetes to fish.

Our findings concerning the effects of stress on larval migrans of *E. tubifex* in yellow perch recommend a minimum of stress. Possibly three factors are involved in the migration of the larvae from the body cavity encapsulations into the viscera and musculature. Degeneration of the perch flesh and a rise in temperature after the fish are removed from water are highly coorelated with the number of larvae recovered burrowing through the fish. This helps account for the problems sports and commercial fishermen experience in filleting yellow perch. If the fish are allowed to remain

semi-cold on ice or in water the larvae after a few hours will still begin to exit the encapsulations as the flesh degenerates and the ice melts. Keeping the fish very cold or cleaning the guts from the fish as soon as possible would reduce one's chances of finding red worms in the fillets. A recent report of human infection with *Eustrongylides* would suggest thorough cooking of the fillets before consumption.

Much follow-up research is yet needed in order to understand the impact of *Eustrongylides tubifex* on Lake Erie fishes. The transmission cycle needs to be completed, i.e. the development of *E. tubifex* in the first intermediate host. The length of development of the larvae in the fish is unknown. The possible role of transport fish hosts operating in maintaining eustrongylidosis is not fully understood.

The main reservoir bird hosts maintaining infection levels in Lake Erie remains unknown. We are beginning to check resident heron populations. Future checks of migrating duck populations, in particular loons and mergansers, are highly desirable.

Our investigations into the pathology caused by the worm in fish and birds is of a preliminary nature at this point in time. Moderate to severe host reactions to the presence of the worm indicate pathology. Much research will be aimed at this aspect this coming year. Also planned for the future are investigations of mammalion response to an experimental *Eustrongylides tubifex* infection.

In the coming year plans for publication of part of our results are being made. We plan to publish a redescription of the species *Eustrongylides tubifex*. Also planned are notes on the pathology of the worm to the fish *Perca flavescens* and on the finding of a natural adult infection in *Mergus serrator*.

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