THE UNIVERSITY OF MANITOBA

EXPERIMENTAL INFECTIONS OF *CYCLOPS BICUSPIDATUS THOMASI* FORBES AND SALMONID FISH WITH THE TAPEWORM, *TRIAENOPHORUS CRASSUS* FOREL: GROWTH, DIFFERENTIATION, HISTOPATHOLOGY AND HOST MORTALITY

by

Ronald B. Rosen

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT TO THE REQUIREMENTS FOR

THE DEGREE OF

DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

WINNIPEG, MANITOBA

November, 1983

EXPERIMENTAL INFECTIONS OF *CYCLOPS BICUSPIDATUS THOMASI* FORBES AND SALMONID FISH WITH THE TAPEWORM, *TRIAENOPHORUS CRASSUS* FOREL: GROWTH, DIFFERENTIATION, HISTOPATHOLOGY AND HOST MORTALITY

by

Ronald B. Rosen

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

TABLE OF CONTENTS

ABSTRACT

Experimental infections of Cyclops bicuspidatus thomasi, whitefish, Coregonus clupeaformis, and rainbow trout, Salmo gairdneri, with Triaenophorus crassus were conducted to assess: 1) mortality of infected cyclopids 2) factors affecting the growth, differentiation and infectivity of the procercoid 3) the growth, differentiation and migration of plerocercoids in fish and 4) the pathology due to these plerocercoids. Over 80% of C. b. thomasi infected with T. crassus died after 28 days, and a decrease in the mean intensity over time indicated that this mortality was related to parasite numbers. Procercoid size, development and infectivity were retarded at higher infection intensities. Procercoids were larger in adult female cyclopids than in adult males, and at 15° than at 23°C. Plerocercoids in whitefish grew rapidly during the first 60 days of infection. Worm movement up to Day 30 post-infection (PI) resulted from a series of peristaltic waves along the length of the worm. Plerocercoids penetrated through the stomach, pyloric caeca and anterior small intestine into the body cavity by 20 hours PI and into the muscle by Day 5 PI. Worms underwent a variety of migration patterns in the muscle and were wound extensively through this tissue by Day 60 PI after which they coiled upon themselves. The presence of the plerocercoid elicited a chronic inflammatory response in the host which was characterized by: 1) hemorrhaging and infiltration of inflammatory cells into vacated muscle lesions 2) transformation of lesions into granulomas by Day 60 PI and 3) the formation of a capsule around worms by Day 70 PI. Capsules containing live worms were recovered up to three years PI. There was no difference in the rate of growth and liver glycogen between infected and uninfected fish. The development and migration of plerocercoids in

rainbow trout were similar to that in whitefish, but worms occurred more often in non-muscular sites in the former host and were not encapsulated by Day 75 PI. Mortality of infected trout was higher (45%) than in whitefish (21%), but occurred during the same period of time (i.e., 48-59 Days PI).

ACKNOWLEDGEMENTS

Numerous people have contributed to making graduate work at the University of Manitoba both successful and enjoyable. Foremost among these is my advisor Dr. T. A. Dick who instilled in me a tough work ethic without which this thesis could not have been completed. In addition I thank him for his patience, support and constructive criticism. My office mates, B. Poole and A. Shostak, have seen me through both bad and good times, and have contributed a great deal to the success of this thesis. I acknowledge E. Huebner and his graduate students, W. Sigurdson and A. Watson, for contributing to my knowledge of histological technique and for their continuous support. Special thanks are in order for my committee, Dr. T. Galloway, Dr. E. Huebner, Dr. H. Welch and Dr. L. Margolis, who provided excellent criticism during the course of my research and on the writing of this thesis. I would also like to thank Dr. F. Ward for his advice with regard to this work. I acknowledge the University of Manitoba for my University of Manitoba Fellowship. Finally, I would like to thank my wife, Nicki, who has sacrificed much so that I might obtain this degree.

LIST OF TABLES

LIST OF FIGURES

CHAPTER III

LIST OF APPENDICES

GENERAL INTRODUCTION

Triaenophorus crassus Forel is a pseudophyllidean cestode which is found as an adult in the intestine of the northern pike, Esox lucius L. The taxonomic history of this tapeworm is outlined in Appendix I. The life cycle of T. crassus includes cyclopids as first intermediate hosts and salmonid fish as second intermediate hosts. The presence of the plerocercoid stage in the muscle of one second intermediate host, the lake whitefish, Coregonus clupeaformis (Mitchill), has led to rejection of heavily parasitized fish for export from Canada. An effort was made starting in the 1940's to control this tapeworm due to the economic loss to the whitefish commercial fisheries in Canada (Miller 1952). Considerable information on natural infections of cyclopids (Arnason 1948; Watson and Lawler 1965) and fish (Miller 1945a, 1945b and 1952) with T. crassus was obtained during the course of studies designed to control this parasite. In addition, experimental infections of copepods were conducted to evaluate the susceptibility of first intermediate hosts in Canada (Price 1958; Watson and Price 1960) and to describe the growth of the procercoid in the first intermediate host (Miller 1943; Lawler and Watson 1963). Little work has been conducted in North America or in Europe on factors influencing procercoid size and development (Miller 1943; Michajlow 1953), and no studies have quantitatively evaluated factors influencing procercoid infectivity and cyclopid mortality. Similarly, considerable gaps in knowledge are also present concerning the nature and timing of events associated with plerocercoid growth, migration and life span, and the host pathology resulting from worm migration and growth. Finally, reports on experimental infections of fish with pseudophyllidean plerocercoids are few and limited in scope, and

knowledge acquired from the T. crassus system may be applicable to other genera in the Order Pseudophyllidea.

The primary objectives of this study were to;

1) evaluate mortality of C. b. thomasi infected with T. crassus
2) assess the effect of procercoid numbers on cyclopid survival
3) identify factors which affect procercoid size, development (=differentiation) and infectivity to the second intermediate host
4) describe the location and orientation of procercoids within C. b. thomasi
5) describe the growth, differentiation and migration of the plerocercoid of T. crassus in whitefish fry
6) describe the progression of the host response in infections of whitefish fry
7) assess the life span of plerocercoids in experimental infections of whitefish
8) assess the effect of T. crassus on the health of whitefish fry (i.e., mortality, growth and liver glycogen)
9) evaluate the suitability of rainbow trout, Salmo gairdneri, as an experimental host for T. crassus plerocercoids
10) assess the implications of stocking rainbow trout into T. crassus infected waters

CHAPTER I

DEVELOPMENT AND INFECTIVITY OF THE PROCERCOID OF *TRIAENOPHORUS CRASSUS* AND MORTALITY OF THE FIRST INTERMEDIATE HOST

Introduction

Mortality of cyclopids infected with tapeworm procercoids and procercoid infectivity are poorly documented in the Order Pseudophyllidea. This is apparent in the genus Triaenophorus in which it has been observed that cyclopids infected with procercoids have reduced activity (Miller 1943), but mortality of infected cyclopids has not been clearly demonstrated (Michajlow 1953; Price 1958; Watson and Price 1960; Kuperman 1973). Effects of parasite crowding and host sex on size and rate of differentiation of worms have been alluded to in the genus Triaenophorus (Miller 1943; Michajlow 1953; Lawler and Watson 1963; Kuperman 1973), but the degree to which crowding affects procercoid infectivity to the second intermediate host is unknown. These factors are essential to our understanding of the transmission dynamics of T. crassus under natural conditions and are best assessed, at least initially, under experimental conditions.

The objectives of this study were to 1) evaluate levels and the pattern of mortality in Cyclops bicuspidatus thomasi infected with T. crassus, 2) provide quantitative evidence for the effect of host sex and the intensity of infection on the size and rate of differentiation of the procercoid of T. crassus in C. b. thomasi and 3) examine the effect of crowding in the cyclopid on procercoid infectivity to the second intermediate host.

Materials and methods

General methods

1. Culturing coracidia

Spawning northern pike, Esox lucius, were obtained with gill nets during mid-late April 1981 and 1982 at Falcon Lake, Manitoba ($95^{o}20'W$, $49^{o}40'N$). Digestive tracts were removed from fish, placed on ice and examined within 12 hours. Gravid T. crassus were isolated from the small intestine and placed in Petri dishes containing dechlorinated water at $12^{o}C$. Tapeworms were washed twice in water, placed in 150 x 20 mm Petri dishes containing water and held at $8^{o}C$ in a refrigerator without light. Most eggs were shed by the adult tapeworms after 12-24 hours and appeared as a whitish-yellow layer on the bottom of the dishes. Spent tapeworms were removed from dishes while the remaining contents, including all eggs not adhering to the bottom of Petri dishes, were poured into 250-1000 ml Erlenmeyer flasks, and the flasks sealed with cotton plugs. Eggs still adhering to the bottom of the dishes, as a thin layer, were washed into other flasks and kept as separate cultures. These latter cultures provided the earliest hatching coracidia and were the least contaminated by microorganisms. Cultures were incubated at $8^{o}C$ in a refrigerator in the dark or at $15^{o}C$ in a controlled environment room with a 12 hr./12 hr. light-dark cycle. Background hatch of coracidia was determined every two days by averaging three 0.1 ml aliquots from the top, middle and bottom of each flask (Appendix II). Half the volume of water was decanted from each flask on these days and replaced with fresh aerated water. This was done to reduce the growth of organisms (i.e., bacteria, ciliates, rotifers, etc.).

2. Culturing Paramecium and maintenance of cyclopids

Dry alfalfa, shredded in a blender, was transferred to an Erlenmeyer flask containing autoclaved water and a magnetic stirring bar. The flask was placed on a hot plate set at medium heat and stirred slowly for four hours. The resulting broth was cooled prior to pouring into a large glass jar partially filled with autoclaved water at room temperature (15^{o}-21^{o}C). A small volume of Paramecium from a stock culture was introduced into the jar and the culture was maintained at room temperature. One-half the volume of this culture was removed every 30 days and replaced with an equal volume of fresh broth and autoclaved water. This replenishment of nutrients increased the growth of Paramecium.

Cyclops bicuspidatus thomasi were collected during the spring with a Wisconsin net from Ponds I and III at the Fort Whyte Nature Center, Manitoba (97^{o}20'W, 49^{o}50'N). All samples were initially screened for natural infections of larval tapeworms and other parasites by removing 50 individuals from the sample. These specimens were fixed in 70% EtOH for one hour, hydrated to water, stained in a 15:1 concentration of water-acetocarmine for 12 hours, dehydrated to 100% EtOH, cleared through a series of increasing concentrations of methyl-salicylate and mounted in permount. No infections were noted in these samples. Cyclopids were concentrated by pouring the pond samples through a container fitted with a 37-47uM mesh cloth screen The container was then inverted over a Petri dish and the cloth screen washed with a squirt bottle. Cyclopids were pipetted from the Petri dish into a nine-celled spot plate and examined with a Wild M-3 dissecting microscope. Adult C. b. thomasi were removed from these samples with a fire-polished pipette and placed individually into 10 x 75 mm test tubes filled with 2 ml of water or 4,5 and 6 dram vials filled with 2 or

7.5 ml water. This eliminated the problem of predation between cyclopids. Cyclopids were held in a refrigerator without light at 8°C prior to exposure to coracidia and fed 0.05-0.10 ml aliquots of approximately 50 Paramecium every two-four days.

3. Culturing Artemia and maintenance of whitefish and cisco

Between 5.4 - 8.1 g of Artemia eggs purchased from Ward's Natural Science Establishment, Rochester, New York and Monterey, California (Lot 87W51 01), were placed in anErlenmeyer flask containing 140 g of Instant Ocean (Aquarium Systems, Mentor, Ohio) dissolved in 4000 ml of water. A 100W bulb supplied heat and an airstone aeration to the cultures. Newly hatched Artemia were present within 36-48 hours. Growth of algae on the sides of the flasks developed over time and seemed to enhance the hatching of eggs. Consequently, a moderate amount of algae was left adhering to the flasks during cleaning.

Artemia were harvested by removing air stones from flasks and allowing unhatched eggs to accumulate at the surface of the flask for five minutes. The contents of the flask were siphoned through a 150-200uM mesh net. Siphoning was terminated when the layer of unhatched eggs approached the level of the siphon tube opening. This was important, since feeding whitefish, Coregonus clupeaformis, and cisco, Coregonus artedii, fry large numbers of unhatched eggs caused blockage of the gastrointestinal tract and death. The concentrated Artemia were resuspended in a beaker containing 300 ml of water. Approximately equal portions of Artemia were poured directly from the beaker into tanks for general maintenance of fish. Hatched Artemia were suspended with the aid of a magnetic stirrer and aliquots removed from the beaker and placed in 30 ml vials when fish were to receive the same

diet. The volume of Artemia was proportional to the number of fish in a particular group and ranged from 1-2 ml (i.e., approximately 750-1500 nauplii/fish). Prior to the introduction of Artemia into a tank, water was shut off for a one-four hour period to prevent flushing of nauplii from the tank. This daily feeding regime brought about a four-fold increase in size of control fish during the four month experiments and was therefore considered a growth diet.

Newly-hatched whitefish fry, still in the yolk sac stage, were obtained from the provincial fish hatchery, Grande Rapids, Manitoba ($99^{o}25'W$, $53^{o}10'N$) and from Southern Indian Lake, Manitoba ($99^{o}00'W$, $56^{o}40'N$). The latter location was also the source for cisco fry. All fish were held in 68 or 189 litre tanks with a slow constant flow of $12^{o}C$ water, moderate aeration, an air temperature of $15^{o}C$ and a 12 hr./12 hr. light-dark cycle (8:00 A.M. - 8:00 P.M.). Fish were fed daily between 7:00 and 9:00 A.M. on a combination of Tetramin and Artemia for the first month, then converted to Artemia only. Tank bottoms were cleaned every second day with a siphon modified to prevent loss of fry. Daily temperatures were taken at feeding time.

Experimental

Experiment I

Mortality of C. b. thomasi infected with T. crassus was evaluated and factors contributing to host mortality and the prevalence and mean intensity of infection (Margolis et al. 1982) were determined. Eight groups of 100 cyclopids were selected using a random numbers table. Thirty 0.05 ml aliquots from the supernate of a five day old culture of eggs held at $15^{o}C$ contained a mean number of

58 coracidia/0.05 ml. Three groups of 100 cyclopids were exposed to 58 coracidia/cyclopid (L = low level exposure) and three groups of 100 cyclopids to 116 coracidia/cyclopid (H = high level exposure). Two hundred unexposed cyclopids served as controls. Exposed and control groups were held at 15°C with a 12 hr./12 hr. light-dark cycle and fed Paramecium. Mortality of cyclopids in the control and exposed groups was recorded every 24 hours for 28 days. Cyclopids dying during the experiment and those still alive at Day 28 post-exposure (PE) were fixed, stained and mounted. Sex, intensity of infection and procercoid differentiation were determined from these cyclopids. [Post-exposure refers to cyclopids exposed to coracidia but not necessarily infected, and is used in calculations of prevalence and the frequency distribution of T. crassus in cyclopids. Post-infection refers to infected cyclopids and applies to all calculations dealing with cyclopid mortality, mean intensity, procercoid size and differentiation].

It was difficult to calculate prevalence and determine mortality of infected cyclopids prior to Day 28 PE because procercoids degenerated rapidly following host death. Consequently, formulae were developed to calculate prevalence of procercoids at Day 1 PE and the % mortality of infected cyclopids at Days 14 and 28 PI in the L and H groups taking into account mortality of controls:

[1] Prevalence Day 1 PE $= \frac{A - (A)\ (C) + B}{D}$

[2] % Mortality Day 14 PI $= \frac{A - (A)\ (C)}{A - (A)\ (C) + A^* - (A^*)\ (C^*) + B}$

[3] % Mortality Day 28 PI $= \frac{A - (A)\ (C)}{A - (A)\ (C) + B}$

where A is the number of dead cyclopids prior to Day N, C is the % mortality of uninfected controls prior to Day N, A* is the number of dead cyclopids between Days N-28, C* is the % mortality of uninfected controls between Days N-28, B is the number of cyclopids alive and infected on Day 28, D is the total number of cyclopids exposed to coracidia and where N = 28 for (1) and (3), and N = 15 for (2).

Chi-square contingency tables were used to test for differences in the prevalence and mortality between the three replicates in the L and H groups when male and female cyclopids were pooled or separated. The replicates in the L and H groups were pooled for the following tests. Chi-square contingency tables were used to test for differences in 1) mortality between the uninfected group and infected cyclopids in exposed groups at Days 14 and 28 PI and 2) the prevalence of procercoids at Days 1 and 28 PE and mortality of infected cyclopids at Days 14 and 28 PI between the L and H groups segregated by host sex and between male and female cyclopids segregated by level of exposure to coracidia.

The mean intensity of infection was calculated from the pooled replicates in the L and H groups. Mean intensity was derived from those infected cyclopids that were alive at Day 28 PI and those that died but had recognizable procercoids when examined between Days 13-28 PI. The mean intensity prior to Day 13 PI was not determined because the decomposition of cyclopids and the small size of procercoids prevented accurate counts. The mean intensity of infection prior to Day 28 PI was calculated by adding the number of procercoids in those cyclopids dying prior to Day 28 PI to the number of procercoids in cyclopids alive at Day 28 PI and dividing the total by the number of infected cyclopids examined.

Differences in the mean intensity of infection due to host sex, level of exposure to coracidia and time were assessed by Student's t and Behren's-Fisher tests. Those cyclopids surviving at Day 13 and Day 28 PE from the L and H groups were pooled and the frequency distribution of T. crassus in these cyclopids tested against a negative binomial and Poisson distribution. Decomposition of some infected hosts dying between Days 13-28 PI prevented a direct count of procercoid intensities at Day 13 PI. Therefore, the frequency of intensity levels of T. crassus procercoids in cyclopids was based on observed and extrapolated values. The extrapolated values for decomposed cyclopids were based on the observed frequency intensity levels and calculated prevalence for each of the four time intervals (i.e., Days 13-16, 17-20, 21-24 and 25-28 PI). For example, if 50% of cyclopids at a given time period, i.e., Days 13-16 PI, were observed to have 2 procercoids, then 50% of the decomposed cyclopids were assumed to have 2 procercoids. Decomposed cyclopids assumed to have died from natural mortality were assigned a value of 0. The estimated frequencies of intensities for each of the four time intervals were summed for intensity levels of 0-11 procercoids and added to the intensities in live cyclopids at Day 28 PI to obtain the frequency of T. crassus in C. b. thomasi at Day 13 PI. The parameter K was determined by iteration for the negative binomial and the fit between observed and expected values was analyzed using chi-square for both the negative binomial and Poisson distribution (Bliss and Fisher 1953; Poole 1974).

Experiment II

Growth curves for T. crassus in C. b. thomasi and information on procercoid size and differentiation were obtained at temperatures of 15°C and 23°C. Two groups of 369 cyclopids were selected using a random numbers

table. The exposure dose of coracidia was determined as in Experiment I using 5 and 15 day old cultures of T. crassus eggs. The two groups of cyclopids were exposed to a mean number of 54 coracidia/ cyclopid. One group was held at 15°C, the other at 23°C, with a light-dark cycle of 12 hr./12 hr. and fed Paramecium. Live cyclopids were randomly selected from each of the groups every second day from 2-12 Days PE and at 14, 21 and 28 Days PE. These cyclopids were fixed, stained and mounted to determine procercoid presence, size and state of development.

Supplementary data for procercoid growth curves, size, differentiation, location and orientation were obtained from Experiments I and III in which temperature and feeding regimes of cyclopids were the same as in Experiment II. Student's t and Behren's-Fisher tests were used to test data from: 1) 21-28 day old infections to determine the effect of host sex and crowding on procercoid size and 2) 14-28 day old infections to determine the effect of temperature on procercoid size. Log transformed data was used as the standard deviation varied directly with the mean. Measurements of procercoids were from specimens fixed and stained in situ, and included length less the cercomer and width at the widest point. Chi-square contingency tables were used to test for differences in the proportion of differentiated procercoids (i.e., formed cercomer and frontal invagination) due to crowding and host sex.

The location (i.e., cephalothorax or abdomen) of 14-28 day old procercoids was recorded along with the intensity of infection in each cyclopid. Procercoid size and state of differentiation were compared between parasites in the cephalothorax and those in the abdomen in cyclopids which had abdominal procercoids. The orientation (i.e., position of the frontal invagination and/or hooks relative to the

anterior-posterior axis of the cyclopid) of 14-28 day old procercoids was also noted. Procercoids with their hooks posterior and frontal invagination anterior were considered to have an anterior orientation, while anterior hooks and a posterior frontal invagination were indicative of posteriorly directed worms. A chi-square test was used to test for possible differences in the number of procercoids oriented anteriad vs. posteriad. A 2 x 5 chi-square contingency table was used to test the effect of parasite numbers on procercoid orientation utilizing cyclopids harboring 1-5 procercoids. Differences between the average height, length and width of the cephalothorax of adult male and female C. b. thomasi were tested with Student's t and Behren's-Fisher tests. Values of $P \leq 0.05$ were considered significant for all tests.

Experiment III

Host and procercoid age, intensity of infection in cyclopids and method of exposure of fish to infected cyclopids (i.e., gastric intubation and natural ingestion) may have influenced the establishment of plerocercoids in fish in pilot experiments (Appendix III). Experiment III was designed to assess the effect of procercoid age and intensity of infection on infectivity of the procercoid to fish. General methods applicable to all fish infections will be discussed in this section, while specific data relevant to an individual group of infections is summarized in Table 1. Ten cyclopids were randomly selected from each group of exposed C. b. thomasi, fixed, stained, mounted and the intensities of infection and differentiation of procercoids determined one hour prior to exposing fish to infected cyclopids. It was assumed that the remaining copepods in a group were infected similarly, and the following calculation determined infection dose:

TABLE 1. Condition of exposure of cyclopids and fish to T. crassus and recovery of procercoids and plerocercoids.

	Cyclopids				Fish			
Expt. No.	Age (Days) Corcacidia Culture	$\bar{X}$ Coracidia /Cyclopid (Vol. H_2O)	Prev.§ Infect. (N) †	$\bar{X}$ Inten. Infect. (Range)	Age (Days) Procer.	$\bar{X}$ Procer. /Fish	Prev. Infect. (N)†	$\bar{X}$ Inten. Infect. (Range)
1w*	7,15,17 (I,8 or 15)‡	52,54,64 (2 ml)	60.0 (805)	2.2 (1-4)	25-27 (G,15)‡	3.4	53.3** (45)	2.8 (1-7)
2w	27 (I,15)	56 (2 ml)	75.0 (75)	5.5 (4-8)	21 (I,15)	98.4	100.0 (2)	9.0 (5,13)
3w	32 (I,8)	80 (2ml)	58.3 (228)	5.4 (3-12)	21 (G,15)	25.6	32.0 (25)	1.3 (1-2)
4w	10 (I,8)	100 (7.5ml)	81.8 (344)	4.3 (1-10)	15 (I,15)	27.3	11.7 (60)	1.6 (1-4)
	10 (G,8)	- (800ml)	60.0 -	4.0 (2-5)	15 (I,15)			
5w	11 (I,8)	134 (7.5ml)	90.0 (288)	5.0 (2-7)	15 (I,15)	18.4	3.3 (60)	1.0 (1)
	8 (I,15)	76 (2ml)	100.0 (288)	4.7 (2-6)	14 (I,15)			
6w	12 (I,15)	67 (2ml)	80.0 (192)	3.8 (1-6)	16 (I,15)	11.3	3.7 (54)	1.0 (1)
	14 (I,8)	85 (2ml)	100.0 (192)	4.5 (2-9)	14 (I,15)			
7w	8-11 (I,15)	76-106 (2ml)	70.0 (456)	5.0 (1-9)	14-17 (I,15)	14.0	7.4 (54)	1.3 (1-2)
8c*	24 (I,8)	96 (2-7ml)	72.7 (344)	5.4 (2-8)	16 (G,15)	18.0	0 (44)	0
9c	19 (I,8)	88 (2ml)	80.0 (312)	5.5 (1-12)	16 (I,15)	9.7	5.0 (60)	1.0 (1)
	20 (I,8)	69 (2ml)	90.0 (300)	3.4 (1-7)	17 (I,15)			

*w = whitefish; c = cisco.
§ Prevalence at time fish were exposed to cyclopids (see procercoid age).
‡ Number hosts exposed.
†I = individual infections; G = group infections, °C.
**Frequency distribution of T. crassus in whitefish Expt. 1 was tested for fit to the negative binomial (see Appendix IV).

(4) No. Procercoids/Fish =

$$\frac{\text{No. Cyclopids} \times \text{Prev. of Infect.} \times \bar{X} \text{ Inten. of Infect.}}{\text{No. Fish}}$$

One to three month old whitefish and cisco were acclimated to water temperatures of 15-17°C for at least seven days prior to exposure to infected cyclopids. Fish were starved for 48 hours and were either isolated individually into beakers as described by Dick and Rosen (1982), or into groups of 20-25 fish for one hour before exposure to infected cyclopids. A known number of C. b. thomasi were then presented to these groups and within four hours all cyclopids had been consumed. Fish were necropsied or examined histologically between Days 1 - 120 PE to determine the prevalence and mean intensity of plerocercoids.

Results

Prevalence, mean intensity of infection and cyclopid mortality (Expt. 1)

No significant difference ($P > 0.05$) was found in the prevalence of infection at Days 1 and 28 PE or mortality at Days 14 and 28 PI between replicates in the L and H groups when host sex was combined or separated. This justified pooling of replicates in the L and H groups for the following comparisons.

Female cyclopids in the L group had a significantly lower prevalence of infection than females in the H group at Day 1 PE (Table 2). Male cyclopids had a significantly higher prevalence of infection than females in the L group at Day 28 PE when the data in Table 2 were segregated by level of exposure to coracidia. At Day 14 PI, female cyclopids had a greater mean intensity of infection than males in the L and H groups (Table 3). Cyclopids in the H group had a significantly higher mean intensity of infection than those in the L group when segregated by host sex at Day 14 PI (Table 3). The mean intensity was significantly lower at Day 28 PI than at Day 14 PI when data were separated by level of exposure to coracidia and host sex (Table 3). The one exception was female cyclopids in the L group in which the mean intensity was not influenced by time PI. At Day 28 PI, male cyclopids in the L group had a significantly smaller mean intensity of infection than males in the H group (Table 3).

Cyclopid mortality in the control group was significantly lower than in the L and H groups at Days 14 and 28 PI (Table 2). Death of infected cyclopids followed a bimodal pattern, with an initial peak occurring at Days 1-4 PI followed by a larger peak at Days 13-16 PI or 17-20 PI

TABLE 2. Mortality of infected and uninfected cyclopids and prevalence of procercoids in cyclopids by host sex.

Comparison of Treatments	Cyclopid Mort. Day 14 PI	Cyclopid Mort. Day 28 PI	Prev. of Infect. Day 1 PE	Prev. of Infect. Day 28 PE
C*	4/200[@]a (2.0%)	8/200 d (4.0%)		
L*	67/200 a (33.5%)	173/200 d (86.5%)		
C	4/200 b (2.0%)	8/200 e (4.0%)		
H*	105/232 b (45.3%)	204/232 e (87.9%)		
Male Hosts-L	24/101 c (23.8%)	84/101 (83.2%)	101/138 (73.2%)	17/49 (34.7%)
H	38/91 c (41.8%)	78/91 (85.7%)	91/110 (82.7%)	13/28 (46.4%)
Female Hosts-L	43/99 (43.4%)	89/99 (89.9%)	99/155 f (63.9%)	10/63 (15.9%)
H	67/141 (47.5%)	126/141 (89.4%)	141/181 f (77.9%)	15/51 (29.4%)

*C = controls; L = infected, 58 coracidia/cyclopid; H = infected, 116 coracidia/cyclopid.

@Mortality = no. control dead/total controls and no. infected dead/total infected; Prevalence = no. infected/no. exposed; Both mortality and prevalence calculated from formulae on page 9.

Figures followed by same letter are significantly different at $P < 0.05$, chi-square test.

TABLE 3. Comparison of the mean intensity of infection at Day 14 vs. Day 28 PI in cyclopids segregated by host sex and level of exposure to coracidia.

Host Sex and Level of Exposure		Mean Intensity ± S.D.	N #
M-L +	14 *	2.00 ± 1.03 a	52
	28 *	1.24 ± 0.57 a	17
M-H +	14	3.00 ± 2.28 b	46
	28	2.08 ± 1.12 b	13
F-L +	14	2.65 ± 1.33	45
	28	2.00 ± 1.49	10
F-H +	14	3.95 ± 2.64 c	56
	28	1.80 ± 1.08 c	15

+ M = male; F = female; L = 58 coracidia/cyclopid;
H = 116 coracidia/cyclopid.

* Days PI.

Number of hosts.

Figures followed by same letter are significantly different at $P < 0.05$, Student's t test and Behren's-Fisher test.

(Fig. 1). Mortality of male cyclopids in the L group was significantly lower than with males in the H group at Day 14 PI (Table 2), and peak mortality occurred in a later time interval for males in the L group (Fig. 1). Significantly more female cyclopids died than did males in the L group at Day 14 PI when the data in Table 2 was segregated by level of exposure to coracidia. Mortality peaked earlier for female cyclopids than males in the L group, and although peak mortality was the same in the H group for both sexes, it was markedly more skewed for male cyclopids (Fig. 1).

The frequency of procercoids of T. crassus in the L and H groups was: 1) distributed according to the negative binomial at Days 28 PE ($k = .436$, $P > .500$, Fig. 2) but not at Day 13 PE ($k = 1.337$, $P < .005$, Fig. 2) and 2) not distributed according to the Poisson at Day 13 or Day 28 PE ($P < 0.005$, Fig. 2).

FIG. 1. Relative frequency of morality over 28 days for adult male and female C. b. thomasi exposed to 58 coracidia/ cyclopid (L) or 116 coracidia/ cyclopid (H) of T. crassus (Experiment I).

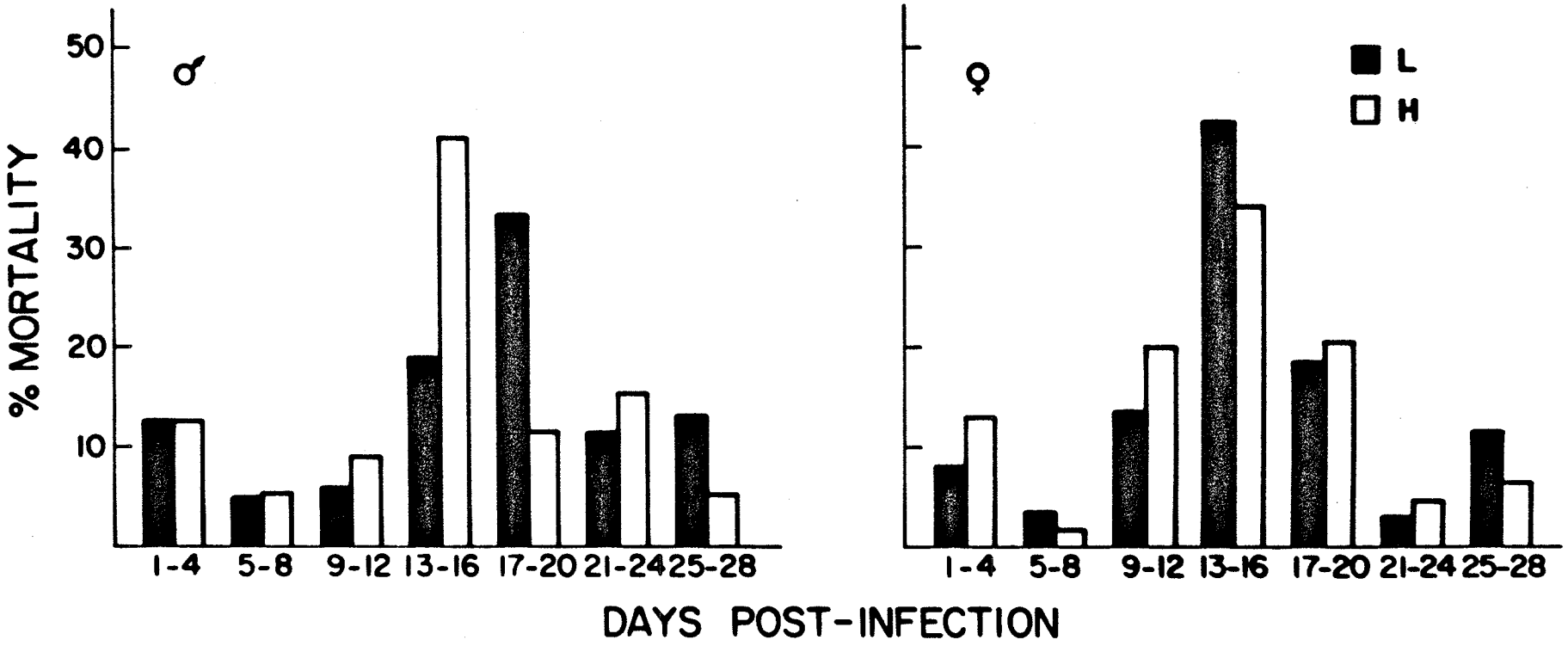
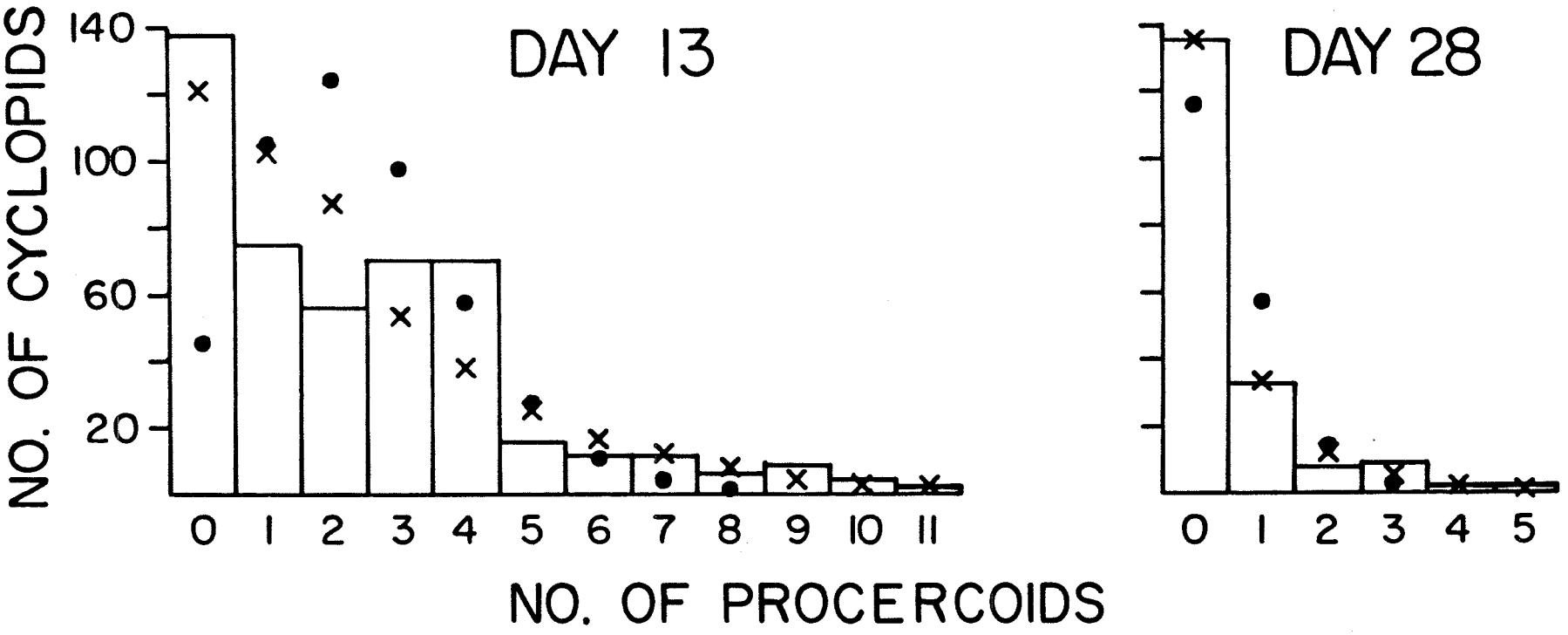

% MORTALITY
50
40
30
20
10
♂
♀
L
H
1-4
5-8
9-12
13-16
17-20
21-24
25-28
DAYS POST-INFECTION

FIG. 2. Frequency distribution of *T. crassus* in *C. b. thomasi* at Day 13 and Day 28 PE. Histograms represent the original data . (X = fitted negative binomial; ● = fitted Poisson distribution).


DAY 13
DAY 28
NO. OF CYCLOPIDS
140
100
60
20
0 1 2 3 4 5 6 7 8 9 10 11
0 1 2 3 4 5
NO. OF PROCERCOIDS

Growth and differentiation of procercoids (Expts. I, II and III)

At both 15° and 23°C procercoids underwent rapid growth between Days 1-16 PI followed by slow growth from 17-28 Days PI (Figs. 3, 4 and 5). Procercoids were significantly smaller in male cyclopids than in females at three levels of infection (Table 4). Female cyclopids, which harbored larger procercoids, had a significantly larger cephalothorax than male cyclopids (Table 5). Lighter infections of procercoids in male and female C. b. thomasi had significantly larger procercoids (Table 4). The mean lengths of procercoids in male cyclopids possessing two vs. three parasites was not significantly different. The effect of crowding on procercoid size could not be tested at higher intensities of infection due to the small number of cyclopids with greater than three 21-28 day old procercoids. Data from 14-20 and 21-28 day old procercoids from Experiments I, II and III indicated that the crowding effect was most dramatic in cyclopids with two-four worms, after which the effect was diminished (Appendices V and VI). Some bias was introduced into the relationship between crowding and procercoid size (Appendices V and VI) as most procercoids in the higher intensities of infection had not attained their maximum size between Days 14-20 PI (Figs. 3 and 4). Procercoid length was significantly greater in female cyclopids held at 15°C than those maintained at 23°C in the three intensities of infection examined (Table 6). The width of procercoids in male cyclopids with two worms was greater at 15°C than at 23°C (Table 6).

Host sex and the number of procercoids fully formed at Days 13-16 and 17-28 PI were not correlated. Therefore, male and female cyclopids were pooled to test the effect of time and crowding on

FIG. 3. Length ($\bar{X}$ ± S.D.) of the procercoid of *T. crassus* in adult male and female *C. b. thomasi* at seven time intervals over 28 days at 15°C. Procercoids were measured from cyclopids possessing 1-5 worms. (* no. procercoids (no. hosts); Bars = 1/2 S.D.).

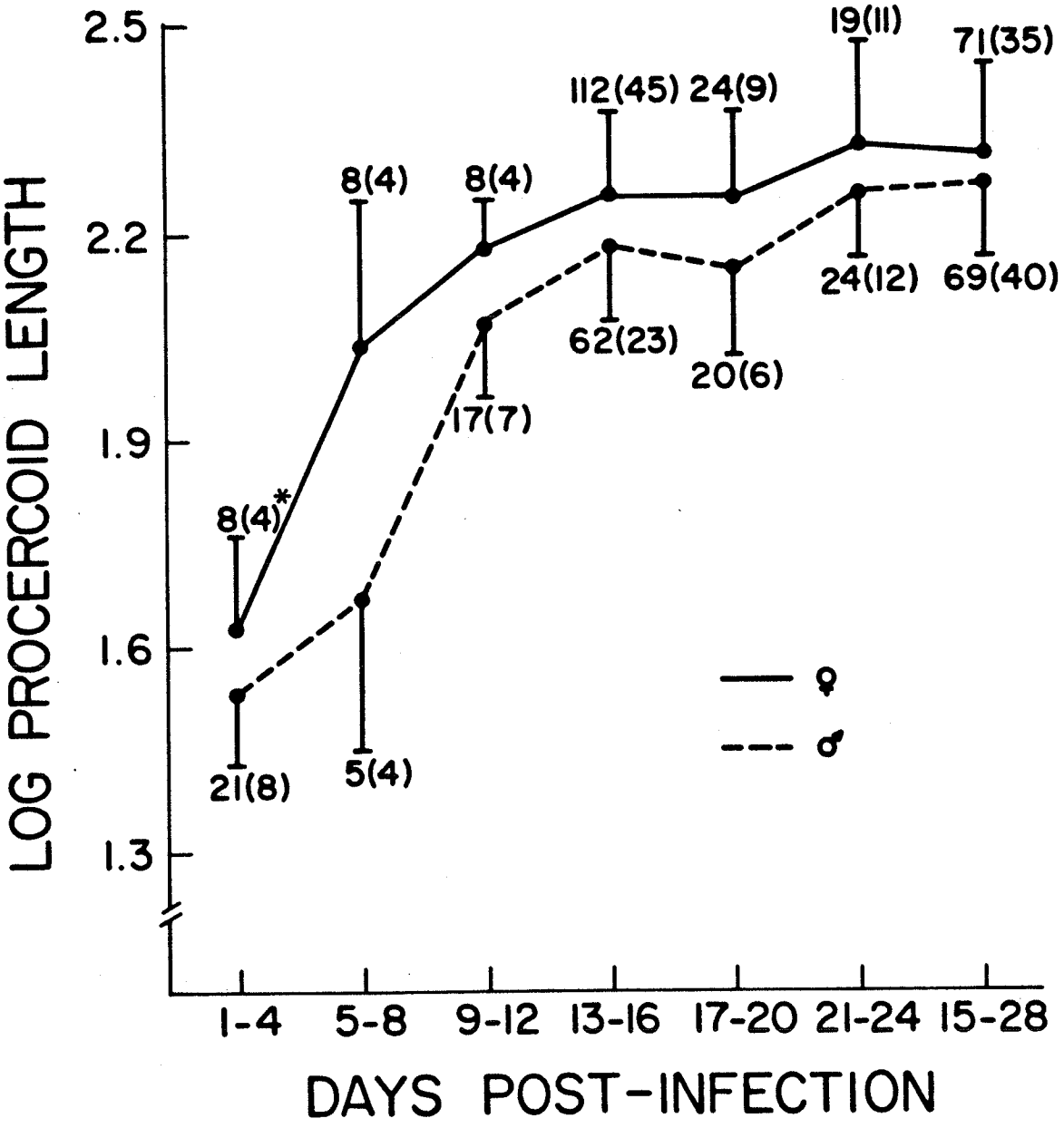

LOG PROCERCOID LENGTH
2.5
2.2
1.9
1.6
1.3
8(4)*
8(4)
8(4)
112(45)
24(9)
19(11)
71(35)
24(12)
69(40)
62(23)
20(6)
17(7)
21(8)
5(4)
♀
♂
1-4
5-8
9-12
13-16
17-20
21-24
15-28
DAYS POST-INFECTION

FIG. 4. Width ($\bar{X}$ ± S.D.) of the procercoid of *T. crassus* in adult male and female *C. b. thomasi* at seven time intervals over 28 days at 15°C. Procercoids were measured from cyclopids possessing 1-5 worms. (* no. procercoids (no. hosts); Bars = 1/2 S.D.).

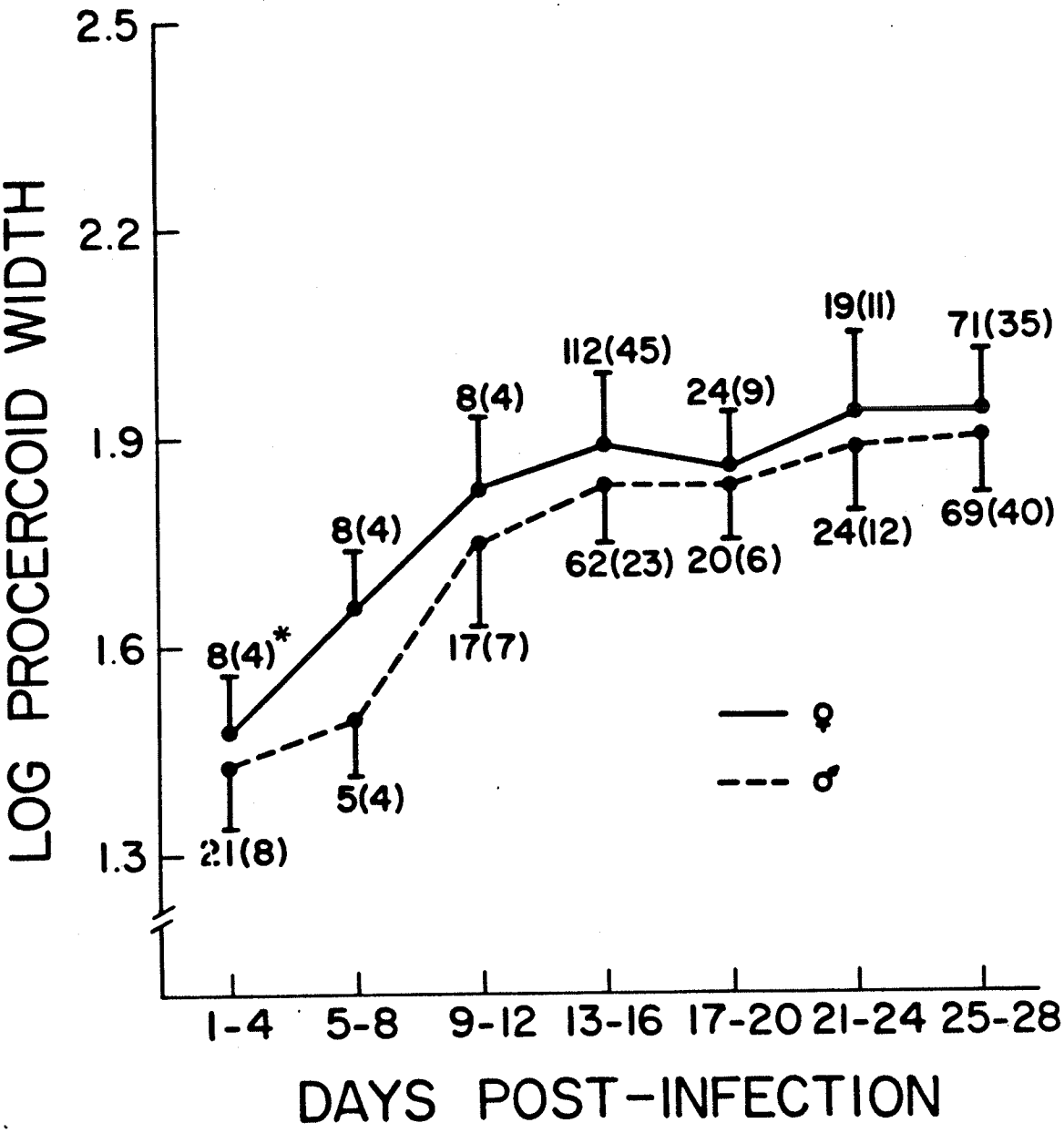

2.5
2.2
1.9
1.6
1.3
LOG PROCERCOID WIDTH
8(4)*
21(8)
8(4)
5(4)
8(4)
17(7)
112(45)
62(23)
24(9)
20(6)
19(11)
24(12)
71(35)
69(40)
♀
♂
1-4
5-8
9-12
13-16
17-20
21-24
25-28
DAYS POST-INFECTION

FIG. 5. Length ($\bar{X}$ ± S.D.) and width ($\bar{X}$ ± S.D.) of the procercoid of *T. crassus* in adult *C. b. thomasi* at six time intervals over 28 days at 23°C. Procercoids were measured from cyclopids possessing 1-5 worms (+ no. procercoids (no hosts); * no. male hosts - no. female hosts; Bars = 1/2 S.D.).

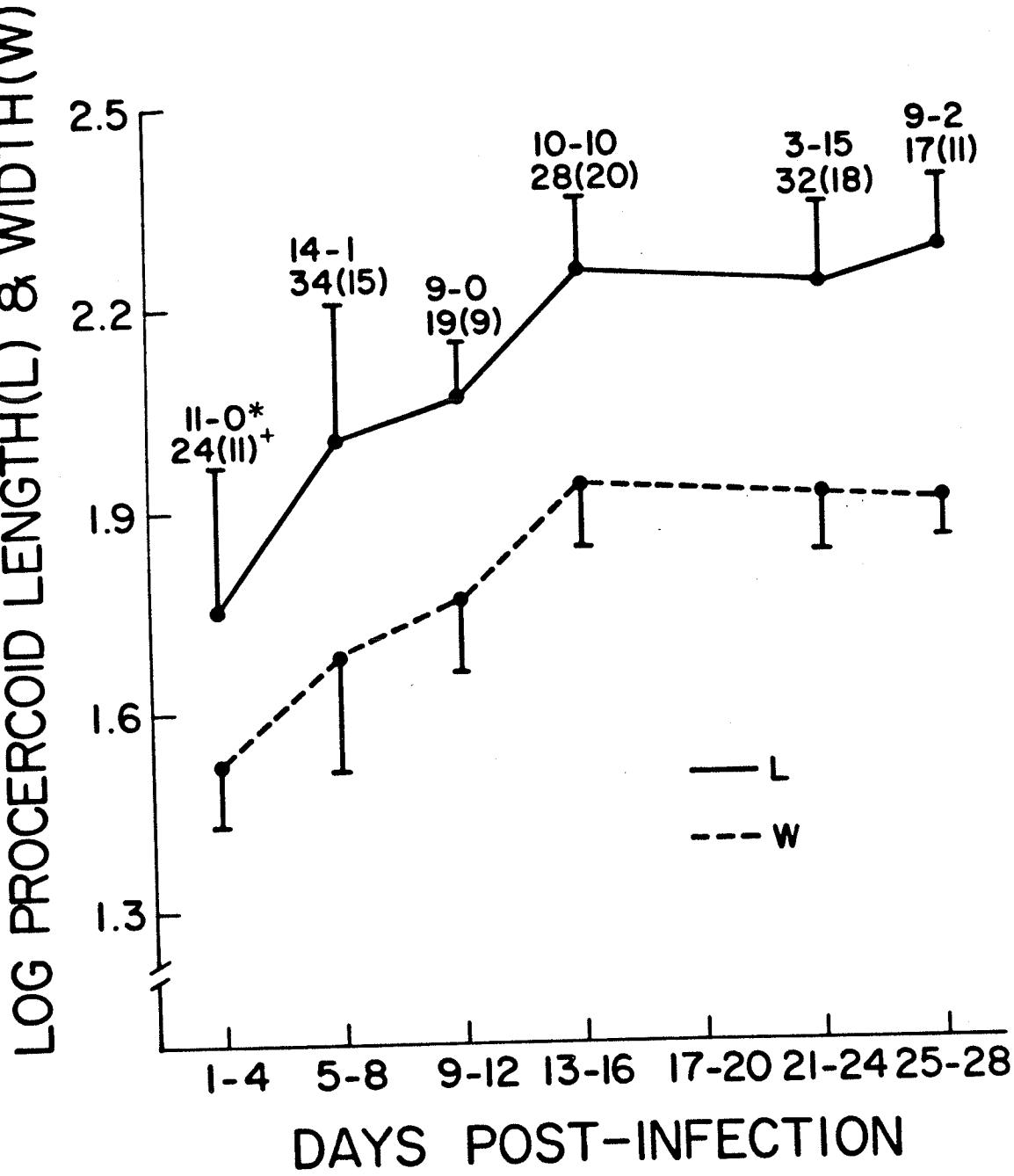

LOG PROCERCOID LENGTH(L) & WIDTH(W)
2.5
2.2
1.9
1.6
1.3
11-0*
24(11)+
14-1
34(15)
9-0
19(9)
10-10
28(20)
3-15
32(18)
9-2
17(11)
L
W
1-4
5-8
9-12
13-16
17-20
21-24
25-28
DAYS POST-INFECTION

TABLE 4. Comparison of $\bar{X}$ length and width of 21-28 day old procercoids in adult male (M) vs. adult female (F) cyclopids. All measurements are in uM.

Inten. of Infect.	Length $\bar{X}$ ± S.D.		Range	N*	Width $\bar{X}$ ± S.D.		Range	N*
1 (M)	233.14±40.55	‡	142-303	29(29)	95.17±11.64	‡	64-112	29(29)
(F)	295.00±37.91		236-374	25(25)	109.52±13.02		79-131	25(25)
2 (M)	188.46±34.60	‡	112-232	22(11)	86.32± 9.43	‡	64-101	22(11)
(F)	229.64±30.60		157-273	14(7)	95.36±11.24		82-120	14 (7)
3 (M)	175.67±32.00	‡	112-228	24(8)	72.71±9.40	‡	49- 90	24 (8)
(F)	198.38±39.38		112-302	24(8)	83.75±9.76		60-101	24 (8)

*Number of procercoids (number of hosts).

‡Sig. at $P < 0.05$, Student's t tests on log transformed data.

TABLE 5. Comparison of the length, height and width of the cephalothorax of adult male (M) and female (F) *C. b. thomasi*. All measurements are in uM.

Cephalothorax		$\bar{X}$ ± S.D.	N*
Length	M	547.59±39.06	51
	F	734.18±54.42‡	45
Height	M	176.75±28.63	40
	F	326.63±53.93‡	24
Width	M	217.18±43.46	11
	F	355.52±32.30‡	21

* Number of cyclopids.

‡ Sig. at $P < 0.05$, Student's t and Behren's-Fisher tests.

TABLE 6. Comparison of the $\bar{X}$ length and width of 14-28 day old procercoids of T. crassus from adult male and female C. b. thomasi maintained at 15°C and 23°C. All measurements are in uM.

Infec. Level	Female ·			Male		
	No. Procer.	Procer. Length @	Procer. Width	No. Procer.	Procer. Length @	Procer. Width
1(15)*	44	277.41±54.10 †	105.77±14.63	37	227.81±42.96	95.35±11.69
1(23)	15	236.13±32.04	110.33± 8.36	13	208.54±29.89	92.15±11.28
2(15)	40	216.48±40.98 †	87.45±15.14	34	180.50±35.79	83.57±10.80 ‡
2(23)	14	171.64±43.57	81.86±10.50	14	162.57±26.43	76.57±10.55
3(15)	54	181.37±42.67 †	76.87±14.74	36	173.36±32.79	70.94±10.13
3(23)	15	148.20±38.52	73.13±13.61	9	159.33±18.06	71.22±11.03

* no. procercoids/host(°C).
† sig. at P < .05, sig. at P < .025, Student's t test.
‡ sig. at P < .05, not sig. at P < .025, Student's t test.
@ Length without cercomer.

procercoid development. Significantly more procercoids were completely differentiated in cyclopids harboring 1-3 procercoids than in cyclopids with 4-5 procercoids at 13-16 and 17-28 Days PI (Table 7). Also, significantly more procercoids were differentiated at 17-28 Days PI than at Days 13-16 PI for infection intensities of 1-3 and 4-5 procercoids per cyclopid.

Procercoids were restricted to the cephalothorax in 97% of the cyclopids examined, whereas the remaining 3% had worms in the cephalothorax and abdomen. The mean intensity of infection was 7.7 ± 2.8 (range 5-13, N=7) and 2.7 ± 2.4 (range 1-16, N=229) in cyclopids with and without abdominal procercoids, respectively, between Days 14-28 PI. The mean length of worms in the abdomen during this time interval was 111.8 ± 27.0 uM, and only 25.0% (2/8) had developed cercomers. In contrast, 14-28 day old procercoids from the cephalothorax of cyclopids with abdominal worms had a mean length of 132.7 ± 38.5 uM, and 69.6% (32/46) had developed cercomers. Procercoids in the cephalothorax were positioned parallel to the longitudinal axis of the cyclopid, and significantly more ($X^2 = 3.84$, $P < 0.05$) worms were oriented anteriad (87.7%) rather than posteriad (12.3%). Conversely, 75.0% of procercoids in the abdomen were oriented posteriorly. The proportion of procercoids oriented anteriad vs. posteriad was independent of the intensities of infection examined ($X^2 = 3.80$, $P > 0.25$).

TABLE 7. Comparison of the proportion of developed procercoids in cyclopid infections with 1-3 vs. 4-5 procercoids segregated by time.

Days PI	Intensity of Infect.	% Procercoids Developed		N*
13-16	1-3	87.7	‡	81
	4-5	64.5		76
17-28	1-3	97.1	‡	136
	4-5	85.0		60

* Number of procercoids.

‡ Sig. at $P < 0.05$, chi-square.

Infectivity of procercoids (Expt. III)

There was considerable variation in the % recovery (i.e., $\left(\frac{\text{no. plerocercoids recovered}}{\text{no. procercoids administered}}\right)$ of plerocercoids from experimentally infected whitefish and cisco. This was apparently related to; 1) procercoid age and intensity of infection in the cyclopid at the time of fish infections and 2) the number of procercoids to which fish were exposed. Whitefish exposed to approximately equal numbers of procercoids from cyclopid groups with a similar mean intensity of infection had a higher prevalence of infection and % recovery when older procercoids were used (Table 1, see experiments 3 and 4). Also notable was the higher prevalence and % recovery in whitefish exposed to older procercoids from lightly infected cyclopids (Table 1, see experiment 1). Whitefish exposed to large numbers of procercoids had a low % recovery of plerocercoids (Table 1, see experiment 2), but whitefish exposed to fewer procercoids had a high % recovery (Table 1, see experiment 1) when procercoids of a similar age were used, but the mean intensity in the cyclopid groups differed.

Discussion

Prevalence, mean intensity of infection and cyclopid mortality

Differences in the level of activity of adult male and female C. b. thomasi may have been responsible for the significant effect of coracidia density on prevalence in female cyclopids but not on prevalence in males at Day 1 PE. Watson and Price (1960) found that male C. b. thomasi consumed more coracidia of T. crassus than adult females over eight hours, and if cyclopids contact coracidia by random collisions as hypothesized by Mueller (1959a) then males may be more active than females. This greater activity of male cyclopids probably reduced the effect of coracidia density resulting in a similar prevalence of infection between males in the L and H experimental groups. The greater prevalence in the slower moving females at a higher exposure dose at Day 1 PE indicated females were affected by coracidia density. Differences in the mean intensity were related to cyclopid sex and exposure dose at Days 14 and 28 PI, but differential mortality between sexes may have also contributed to the mean intensity of infection.

Previous experiments had shown that there was considerable mortality of C. b. thomasi exposed to coracidia of T. crassus (Appendix VII), but it was not clear whether this was due to infection or natural mortality. This study clearly showed a significant mortality of C. b. thomasi infected with the procercoid of T. crassus. The first peak of mortality occurred when coracidia penetrated the body cavity of the cyclopid and was probably due to damage caused during migration. The second and larger peak of mortality coincided with the completion of most of the procercoid growth. Death at this time may be related to mechanical pressure on internal organs or nutritional stress. Procercoids were

observed breaking out of some dead cyclopids and this response could be a direct cause of death or a post-mortem response of the procercoid.

The decrease in the mean intensity of infection over time indicated that host death was related to procercoid number. Differences in the mean intensity of infection did not always result in differences in the level of mortality when cyclopids were compared by sex or exposure dose of coracidia. Similarly, Keymer (1980) indicated that a linear relationship did not exist between host mortality and parasite burden in overdispersed populations of Hymenolepis diminuta in Tribolium confusum. Whatever the exact relationshp between cyclopid mortality and the mean intensity of infection, the final effect at Day 28 PE was a cyclopid population dominated by uninfected and lightly infected hosts whose parasite frequency was described by the negative binomial distribution. These results corroborate the work of Keymer and Anderson (1979) and Tanner et al. (1980) in which a negative binomial distribution was generated for helminths even under the constraints of controlled laboratory conditions. The frequency of T. crassus at Day 13 PE did not fit a negative binomial (overdispersed) or Poisson (random) distribution although it appeared to be overdispersed. Apparently, different frequency distributions occur for T. crassus in C. b. thomasi during the course of the infection, similar to the findings of Keymer (1980) for H. diminuta in T. confusum. In both cases, changes in parasite frequency were attributable to mortality of hosts with large parasite burdens.

Although overdispersed, the range of procercoid numbers/ host was limited (i.e., 0-5 procercoids/cyclopid) in our experimental infections at Day 28 PE, and seemed to reflect the frequency of this parasite in

natural systems. Arnason (1948) and Watson and Lawler (1965) found C. bicuspidatus infected with procercoids of T. crassus or T. nodulosus, and usually each cyclopid had a single procercoid. The low intensity of procercoids recovered in natural systems may result from low coracidia densities and/or host mortality. Cyclopids experimentally exposed to various coracidia densities had mean intensities of infection of 2.2 - 5.5 procercoids/host, with a maximum of 12 procercoids. Little is known on coracidia densities in lake systems, although the apparently clumped distribution of cyclopids infected with Triaenophorus spp. (Watson and Lawler 1965) suggests that coracidia may be similarly concentrated. It is probable that exposure densities used in our experiments were in excess of those in whole lakes. It is also clear that these high coracidial densities produced low experimental intensities of infection and this may help explain even lower intensities encountered in natural systems. A low initial intensity may be further reduced by mortality of infected cyclopids, especially in cyclopids harboring over one procercoid, as shown in these experiments. Loss may be due to death of cyclopids as shown in our experimental infections, or may result from increased predation of heavily infected and less active cyclopids.

Growth and differentiation of procercoids

The growth curves obtained for T. crassus procercoids in this study and previous growth curves constructed for procercoids in the genus Triaenophorus (Vogt 1938; Lawler and Watson 1963; Guttowa and Michajlow 1964), although similar in form, vary in sizes of developing procercoids. This may be due to differences between cyclopid hosts (species, stage used for infections and sex) and experimental design (intensities of infection, temperature and sample size). Host sex, crowding of

procercoids, temperature and time were identified in this study as important factors affecting procercoid size and differentiation.

A decrease in size and number of fully developed procercoids with increased parasite intensities suggested intraspecific competition for space and/or food. The larger size of procercoids in female cyclopids than in male cyclopids may be related to the larger cephalothorax in females and therefore, available nutrients. The smaller size of procercoids in adult male and female C. b. thomasi at 23°C than at 15°C indicated that the higher temperature was above the optimum range for procercoid development. The greater stunting of procercoids in female cyclopids than in males at 23°C when compared to procercoids at 15°C may have been related to factors such as accelerated egg production in female C. b. thomasi, which diverted a greater amount of nutrients away from the worm at 23°C than at 15°C. More procercoids became fully differentiated with time, especially in crowded infections where nutrients were probably most limited. This corroborates the work of Meyer and Vik (1963) for Diphyllobothrium sebago Ward in Cyclops vernalis Fischer and C. b. thomasi.

Procercoids of T. crassus were found in the abdomen of C. b. thomasi in which the intensity of infection exceeded four procercoids/host. Thus, crowding of procercoids not only reduced parasite size and the rate of differentiation, but also forced a small number of worms into the abdomen. Similarly, Halvorsen (1967) found that there was an increase in the relative number of copepodid and female Cyclops strenuus Fischer with procercoids in their abdomen with increasing parasite numbers. Procercoids in the abdomen were rare in experimental infections, and when encountered, were smaller and often had no formed cercomer or frontal invagination in contrast to those in the cephalothorax of the same host. These abdominal worms probably play little if any role in the transmission of T. crassus if they occur in natural infections of C. b. thomasi.

Infectivity of procercoids

Procercoid differentiation and the number of procercoids/ fish were factors which affected the % recovery of plerocercoids in this study. The high mean intensity of infection of procercoids in most of the cyclopid groups indicated that both size and state of differentiation of procercoids in these hosts were adversely affected by crowding. When the time for procercoid development was extended, even under crowded conditions, there was a higher prevalence of infection in fish and % recovery of plerocercoids. Kuperman (1973) found if mean intensities of Triaenophorus spp. procercoids exceeded five/copepod, only 50% were infective. This may have contributed to the low recovery of plerocercoids in this study. Procercoids were larger and more fully developed in older and lighter (i.e., $\bar{x}$ = 2.2 procercoids/cyclopid) infections of C. b. thomasi, and this resulted in more plerocercoids per fish. Although our experiments showed that the degree of procercoid differentiation influenced the number of plerocercoids recovered, it is questionable whether size was a factor since Kuperman (1973) suggested that stunted procercoids were infective as long as they were completely differentiated. Unfortunately, it is not clear if procercoid viability was determined by Kuperman (1973). Finally, the number of procercoids to which fish were exposed affected the % recovery of plerocercoids. Kuperman (1973) suggested that a host response limited the number of Triaenophorus spp. plerocercoids which established when large numbers of infected cyclopids were exposed to fish. Further work will be required to substantiate the existence of such a response.

It is well known that parasite transmission is dependent on many ecological factors. The importance of crowding on differentiation of

larval forms is not well understood and usually overlooked as a factor in transmission. This study has clearly shown that intensity of infection of T. crassus in C. b. thomasi affects cyclopid survival and procercoid growth, differentiation and infectivity. Cyclopids with one procercoid are more likely to survive and have a greater proportion of fully differentiated procercoids than cyclopids with heavier infections. Thus, low intensity infections of cyclopids appear to be an important adaptive strategy for transmission of T. crassus to coregoniid fishes.

CHAPTER II

DEVELOPMENT AND MIGRATION OF PLEROCERCOIDS OF *TRIAENOPHORUS CRASSUS* AND PATHOLOGY IN EXPERIMENTALLY INFECTED WHITEFISH, *COREGONUS CLUPEAFORMIS*

Introduction

Information on the procercoid-plerocercoid tranformation of pseudophyllidean cestodes in fish has been derived largely from natural infections. This has led to speculation on the nature and timing of events associated with plerocercoid growth, differentiation, migration and life span, and the host pathology resulting from worm migration and growth. Pseudophyllidean plerocercoids have been reported for Diphyllobothrium spp. (Janicki and Rosen 1918; Kuhlow 1953; Vik 1957; Meyer and Vik 1963; Bylund 1972 and 1975), Schistocephalus solidus (Clarke 1954-55; Orr and Hopkins 1969) and Triaenophorus spp. (Rosen 1918; Vogt 1938; Kuperman 1966; Dick and Rosen 1982) from experimental infections of fish, but these studies are limited in scope.

Triaenophorus crassus plerocercoids are usually located in the muscle of whitefish, Coregonus clupeaformis (Mitchill) and C. artedii Lesueur. Considering its economic importance to the whitefish commercial fishery, there is little information on the growth, differentiation, migration and pathology of T. crassus in whitefish. Furthermore, there are conflicting reports on the effect of T. crassus on whitefish growth (Miller 1945 a; Lawler 1952; Petersson 1971), although it is known to cause mortality in experimentally infected whitefish fry (Dick and Rosen 1982). The objectives of the present research were to; 1) describe the sequence of developmental and pathological events which occurred during the differentiation of the plerocercoid of T. crassus in whitefish and 2) determine the effect of this cestode on the health of its second intermediate host.

Materials and methods

Whitefish fry used in these experiments were obtained from the Grande Rapids Hatchery, Manitoba, and Southern Indian Lake, Manitoba. These fish were assumed to be genetically similar as no differences in susceptibility of these two fish stocks to T. crassus were noted.

Experiment I

Growth of infected whitefish fry and plerocercoid growth and differentiation were evaluated in this experiment. The methods for processing adult tapeworms, culturing coracidia, infecting Cyclops bicuspidatus thomasi and maintenance of fish have been described in Chapter 1. One-hundred whitefish fry were divided into five groups of 20 fish/tank and acclimated to a water temperature of 15-17^{o}C for two weeks. Five fish were randomly selected from each tank, sacrificed and their fork lengths recorded one hour prior to exposing the remaining fish to infected cyclopids and Artemia. This was done to ensure that groups of fish did not differ significantly in size. Three tanks of whitefish (45 fish) were then exposed to three procercoids/fish following the methods in Chapter 1, and two tanks of fish (30 fish) were exposed to Artemia only and served as controls. Each group of fish was fed an equivalent volume of Artemia during the experiment which was periodically adjusted upon death of infected fish or removal of infected and control fish.

Four or five infected fish from the exposed group and ten fish from the control group were sacrificed at Days 30, 55 and 80 post-infection (PI). Mean fork lengths were recorded and compared between control and infected fish at each time interval with Student's t and Behren's-Fisher tests. All livers from infected fish and five livers from control fish at each time interval were removed and fixed in

Gendre's fluid, routinely processed for paraffin embedding and serially sectioned at 10 uM.. Control slides were treated with diastase of malt (Fisher Scientific) and all slides were stained with Periodic Acid-Schiff Method for demonstration of glycogen (Humason 1979). Liver glycogen was assessed by selecting three slides with not less than three sections, from different areas of the liver of each fish. These sections were compared to diastase treated sections to determine the presence of glycogen, and then assigned a qualitative value ranging from one (low glycogen) to three (high glycogen) by three different assessors. These values were averaged for control and infected fish at 30, 55 and 80 Days PI.

Plerocercoids removed from infected fish which died or were sacrificed were placed in physiological saline. Drawings of plerocercoid movement were made and the distance moved recorded. Plerocercoids were then fixed in hot FAA, stained, mounted on slides, their lengths determined and the degree of development noted. Plerocercoid lengths in this experiment and from Experiment II were combined and fitted to a logistic growth curve (Poole 1974). The asymptote was estimated from the lengths of 1.5 - 3 year old plerocercoids in Experiment III.

Experiment II

This experiment assessed the differentiation and migration of the plerocercoid of T. crassus in whitefish and the pathology associated with the infection. A total of 255 fish were divided into six groups of 54 or fewer fish and were acclimated to a water temperature of 15-17^{o}C for at least seven days. Fish were exposed individually to infected cyclopids as described by Dick and Rosen (1982) or in groups as in Experiment I at exposure doses ranging from 11-98 procercoids/fish.

Fish were sacrificed at selected times (i.e., 20 hrs. and 5, 17 22, 30, 35, 40, 50, 60, 63, 66, 70, 80, 90, 100, 110 and 120 Days PI) and fixed whole in Bouin's or in a mixture comprised of 1% paraformaldehyde, 2.5% gluteraldehyde, 0.1M cacodylate buffer (pH 7.2) and 0.05% $CaCl_2$. Twenty-five infected fish fixed in Bouin's were routinely processed for paraffin embedding and serially sectioned at 5-10 uM on a rotary microtome. Sections were stained with hematoxylin-eosin, PAS-hematoxylin, Mallory's trichrome (Humason 1979) and picro-sirius red (Puchtler et al. 1973). Three infected fish fixed in the aldehyde mixture were cut into five transverse sections and embedded in epon-araldite. Sections from these blocks were cut at 1-2 uM with a JB4 Sorval microtome and stained with 0.1% toluidine blue. Infected whitefish which died during the experiment were necropsied and plerocercoids recovered, fixed, stained, mounted and their lengths determined.

Experiment III

Plerocercoid life span and capsule structure in older whitefish infections were evaluated in this experiment. Capsules were dissected from four experimentally infected fish at 1.5, 2, 2.5 and 3 years PI and either fixed in Bouin's or opened for recovery of plerocercoids. Capsules fixed in Bouin's were routinely processed for paraffin embedding, sectioned at 5-10 uM and stained as in Experiment II. Plerocercoids were fixed in hot FAA, stained, mounted and their lengths determined.

Fully developed capsules were removed from whitefish naturally infected with T. crassus. These capsules were processed in the same manner as those in the experimental infections. In addition, sections from these capsules and from 2.5 year old capsules were stained with alizarine red S for the detection of calcium, but under the limitations imposed by this technique (Humason 1979)

Experiment IV

This preliminary study tested the suitability of the whitefish body cavity as a habitat for T. crassus plerocercoids since worms have been observed in this site in both natural and experimental infections. Plerocercoids were removed from capsules in the muscle of cisco, Coregonus artedii, obtained from Quigly Lake, Manitoba (54^{o} 51'N, 101^{o} 04' W). Fully differentiated plerocercoids (4-5/fish) of unknown age were pipetted into the body cavity through an incision made in the ventral hypaxial muscle of nine 4-5 year old laboratory-reared whitefish packed in ice and anesthetized with MS-222. The incision was then closed with catgut chromic 3/0 USP (B. Broun Melsungen A.G., W. Germany), and the fish placed in well aerated water and held at 12^{o}C. Fish were sacrificed at weekly intervals starting at Day 7 PI. Plerocercoids and associated host tissue were removed, placed in physiological saline and observed with a dissecting microscope. Worms and tissue were then fixed in Bouin's, routinely processed for paraffin embedding, sectioned at 10 uM and stained with hematoxylin and eosin.

Results

Plerocercoid growth, differentiation and movement

Plerocercoids of T. crassus doubled their length every ten days for the first 60 days of infection as determined by the logistic growth model (Fig. 1). The rate of plerocercoid growth declined after 60 days, but plerocercoids continued to increase in length even after two months PI. The length varied more after Day 30 PI for plerocercoids of the same age and even from the same fish (Fig. 1). The length of 1.5 - 3 year old plerocercoids ranged from 74-155 mm.

The scolex region of worms up to 30 days old (Fig. 2A) was differentiated from their posterior end (Fig. 2B) by the presence of a frontal invagination. The scolex of 40 day old worms, in addition to the frontal invagination, had more cells in the parenchyma (Fig. 2C) than in the parenchyma of the posterior end (Fig. 2D). Developing hooks were observed in pockets in the scolex as early as Day 70 PI (Fig. 2E, Table 1). Collagen covered the outer edge of the developing hook, and was most concentrated in the region of the hook proximal to the opening of the pocket (Fig. 2E). Hypertrophied tegument formed a cap over the developing hook (Fig. 2E). Fully-formed hooks were first observed at Day 80 PI, (Table 1) but in some cases were not developed until Day 100 PI (Fig. 2F). The frontal invagination was reduced to a pit in the scolex region of plerocercoids with hooks.

Movement of 19-30 day old plerocercoids in physiological saline is illustrated in Fig. 3. A peristaltic wave, originating in the anterior half of the plerocercoid (Fig. 3A), produced a phallus-like anterior end (Fig. 3B). A second peristaltic wave passed from the anterior end posteriad (Fig. 3B), producing a bulge in the anterior half of the

FIG. 1. Lengths of 68 plerocercoids of *T. crassus* recovered from whitefish held at 15-17°C plotted against time. Curve is calculated from the logistic growth model. (* numbers indicate more than one plerocercoid measurement).

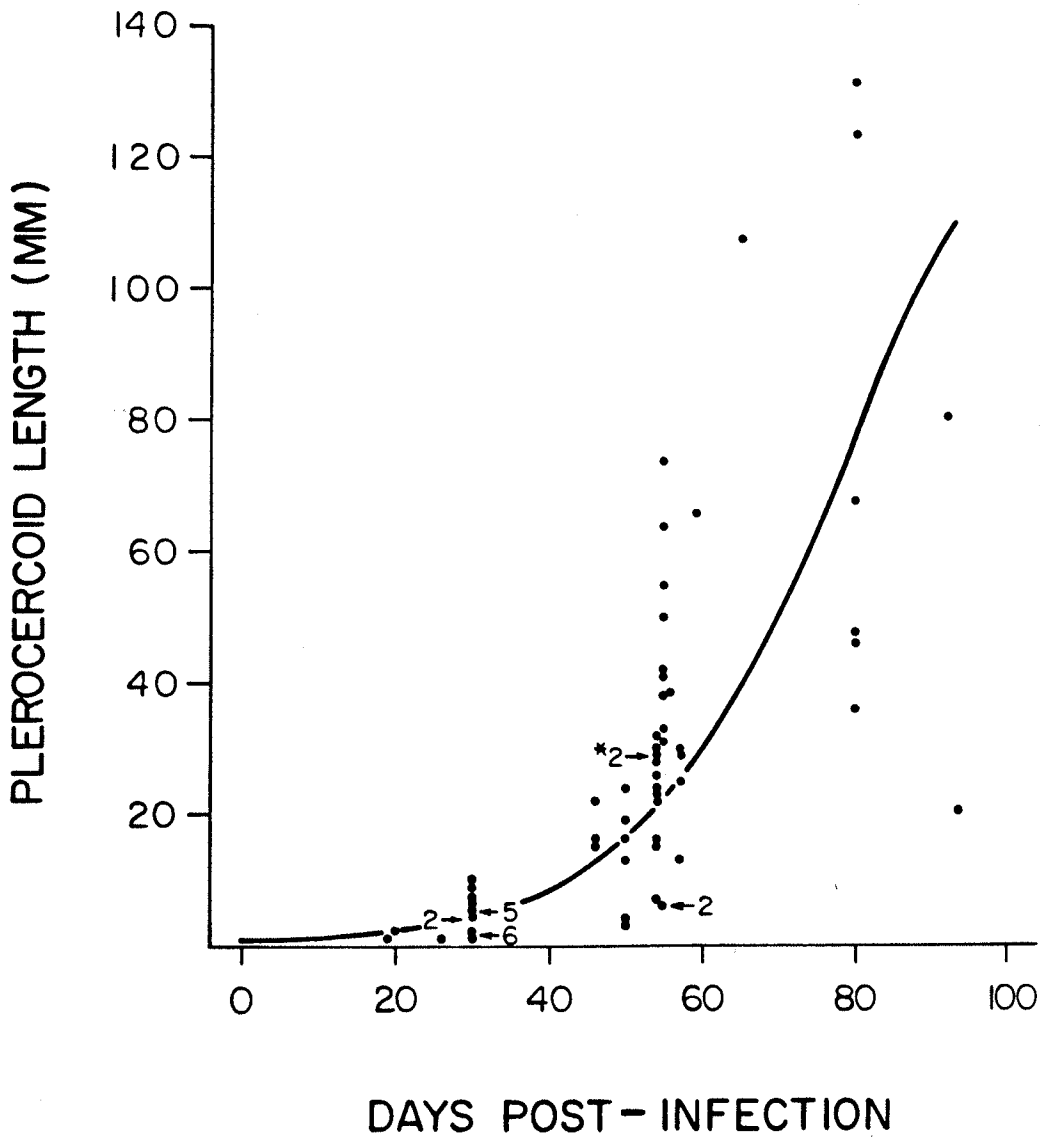

PLEROCERCOID LENGTH (MM)
140
120
100
80
60
40
20
0
20
40
60
80
100
DAYS POST-INFECTION
2
5
6
*2
2

FIG. 2. (A) Section through scolex region of plerocercoid *in situ* at Day 22 PI. Note frontal invagination (arrow). Hematoxylin and eosin (H & E), X 660. (B) Section through posterior region of plerocercoid in A. H & E, X 480. (C) Section through scolex region of plerocercoid *in situ* at Day 40 PI. Large numbers of cells and the frontal invagination (arrow) were present in this region. H & E, X 280. (D) Section through posterior region of plerocercoid in C which contained fewer cells than in scolex region. H & E, X 200. (E) Section through scolex region of plerocercoid in situ at Day 80 PI. Note developing hooks in hook pockets (arrow), collagen tissue and hypertrophied tegument. Picro-sirius red, X 170. (F) Section through scolex region of plerocercoid *in situ* at Day 100 PI. Hooks were fully formed and associated with collagenous tissue on outer edge. Picro-sirius red, X 120. co=collagenous tissue, h=hooks and t=hypertrophied tegument.

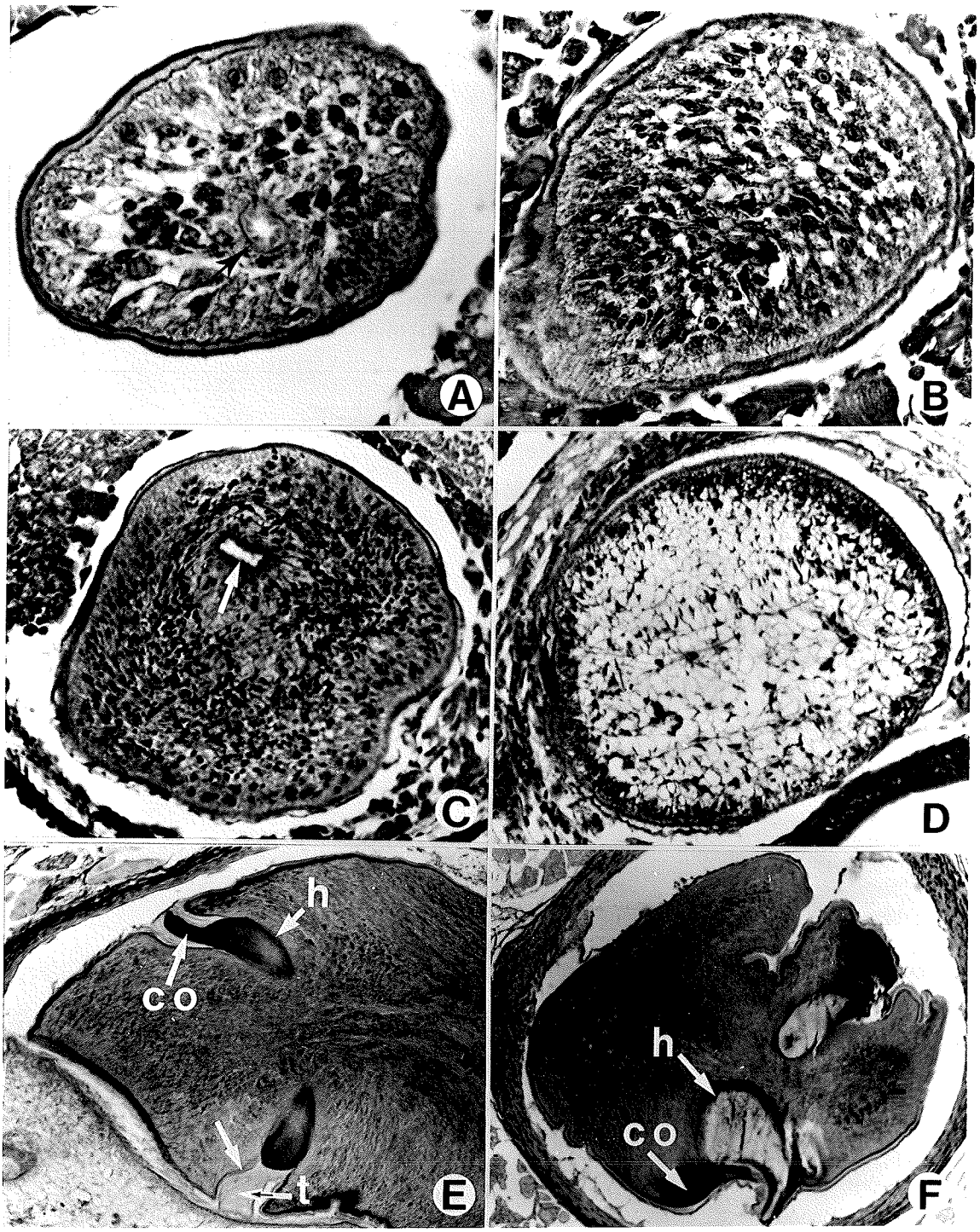
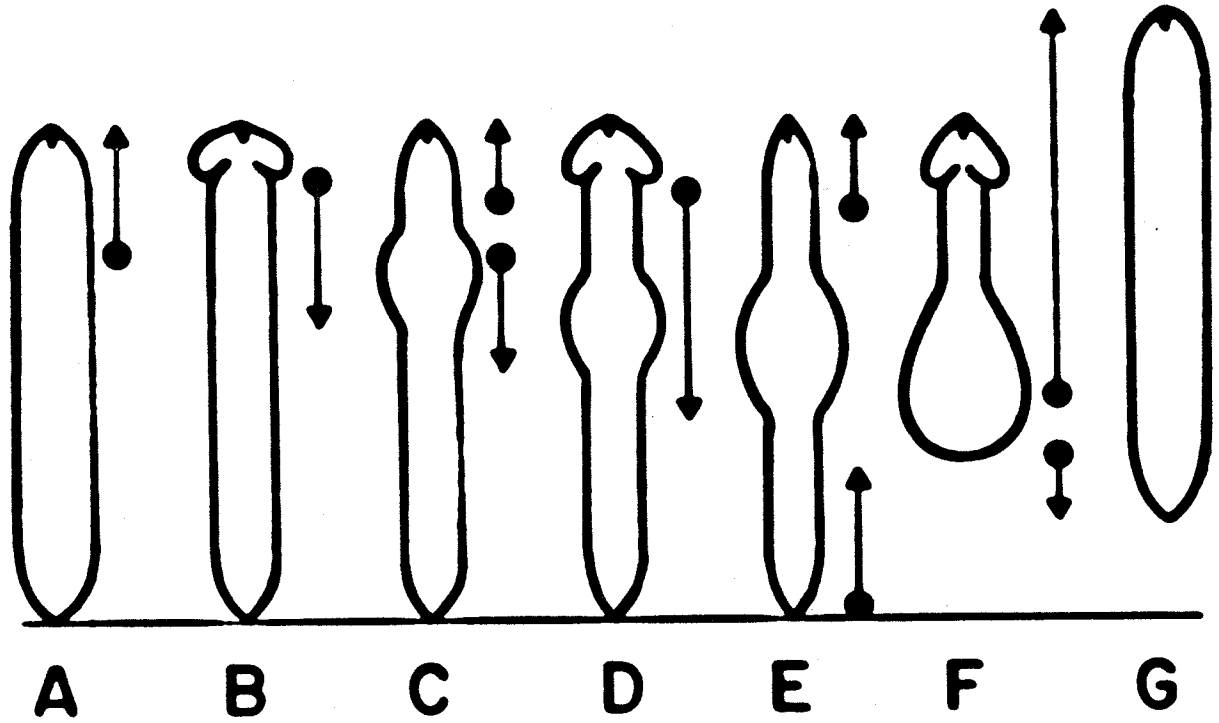

A
B
C
D
h
co
E
h
co
F

TABLE 1. Location of 125 plerocercoids of T. crassus in experimentally infected whitefish held at 15-17°C. Hook development in these worms is also noted.

DAYS PI	N*	LOCATION						HOOKS	
		Gut Wall	Body Cavity	Muscle	Muscle ‡	Body Cavity & Muscle	Body Cavity & Muscle ‡	Developing	Fully formed
0-10	21	6+	13 §	1	0	1	0	0	0
11-20	4	0	0	4	0	0	0	0	0
21-30	23	0	0	23	0	0	0	0	0
31-40	4	0	0	3	0	1	0	0	0
41-50	12	0	0	12	0	0	0	0	0
51-60	37	0	1	28	7	0	1	0	0
61-70	5	0	0	3	2	0	0	1	0
71-80	7	0	0	7	0	0	0	5	2
81-100	5	0	0	4	1	0	0	1	4
> 100	7	0	1	6	0	0	0	0	7

* N = number of plerocercoids.
‡ Plerocercoid penetration through integument.
+ All at 20 hours post-infection.
§ Plerocercoids in body cavity after this time had reentered this site from the muscle.

FIG. 3. Sequence of movements (A - G) of 19-30 day old plerocercoids of T. crassus in physiological saline. Dots on arrow shafts indicate origin of next movement and arrowheads indicate direction of next movement.


A
B
C
D
E
F
G

worm (Fig. 3C). A second, anteriad directed wave (Fig. 3C) resulted in a new phallus-like anterior region while the bulge continued to move posteriad (Fig. 3D). The phallus-like anterior end disappeared as a posterior directed wave was generated (Fig. 3D). This posterior wave continued until it joined the initial bulge at the center of the plerocercoid (Fig. 3E). The posterior part of the plerocercoid contracted and moved anteriad (Fig. 3E) forming a large rounded posterior end (Fig. 3F), while a new phallus-like anterior end was formed (Fig. 3F). The plerocercoid then elongated in a predominatly anterior direction (Fig. 3F) resulting in a net movement forward which ranged from 10-50% of the total length of the elongated worm or 2.5 mm/minute (Fig. 3G). Movement of plerocercoids 40 days or older was restricted to the anterior 5% of the worm, although there was some random contraction and elongation of worms distal to this region. Movement in the anterior region of these worms was characterized by a constant repetition of steps A, B and C in Fig. 3.

Plerocercoid migration

Plerocercoids penetrated into the body cavity of fish by 20 hrs. PI (Fig. 4A, Table 1) in the region of the stomach-pyloric caeca (Fig. 4B) and the small intestine (Figs. 4A and 4C). Degeneration of villi (Fig. 4C) and hemorrhaging (Fig. 4D) were associated with this stage of migration. Plerocercoids were penetrating body wall muscles (Fig. 5A) or were already in muscle by Day 5 PI. Entry into muscle was observed in the region of the stomach and anterior small intestine, although the location of scar tissue during the first 30 days of infection indicated that penetration occurred along the entire length of the body cavity. Worms oriented parallel to muscle fibres up to Day 30 PI

FIG. 5. (A) Section through region of small intestine at Day 5 PI. Plerocercoid is penetrating into ventral hypaxial muscle. H & E, X 190. (B) Section through epaxial muscle at Day 30 PI. The longitudinal axis of the plerocercoid is parallel to the muscle fibers. Note fixation in gluteraldehyde and embedding in epon-araldite reduced shrinkage of muscle. toluidine blue, X 240. (C) Lesion in epaxial muscle at Day 17 PI. Erythrocytes are major cell type in lesion. Note macrophages and pmn leucocytes. H & E, X 640. (D) Lesion in epaxial muscle at Day 30 PI. Note heavy infiltration of macrophages into lesion (arrow) and necrotic muscle. H & E, X 320. bc= body cavity, e=erythrocytes, hm=hypaxial muscle, i=intestine, L=lesion, M=muscle, ma=macrophages, NM=necrotic muscle, P=plerocercoid and pmn=polymorphonuclear leucocytes.

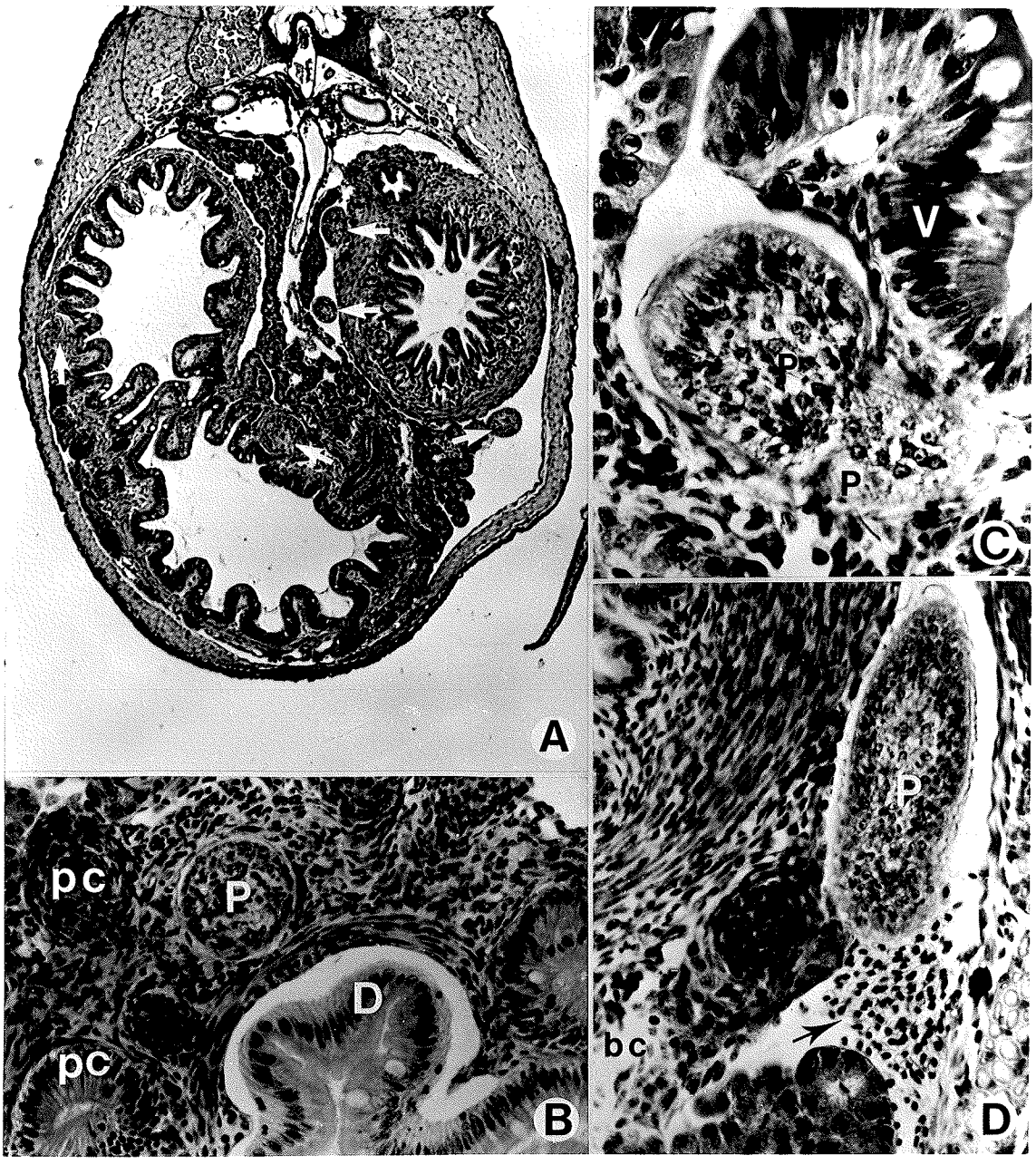
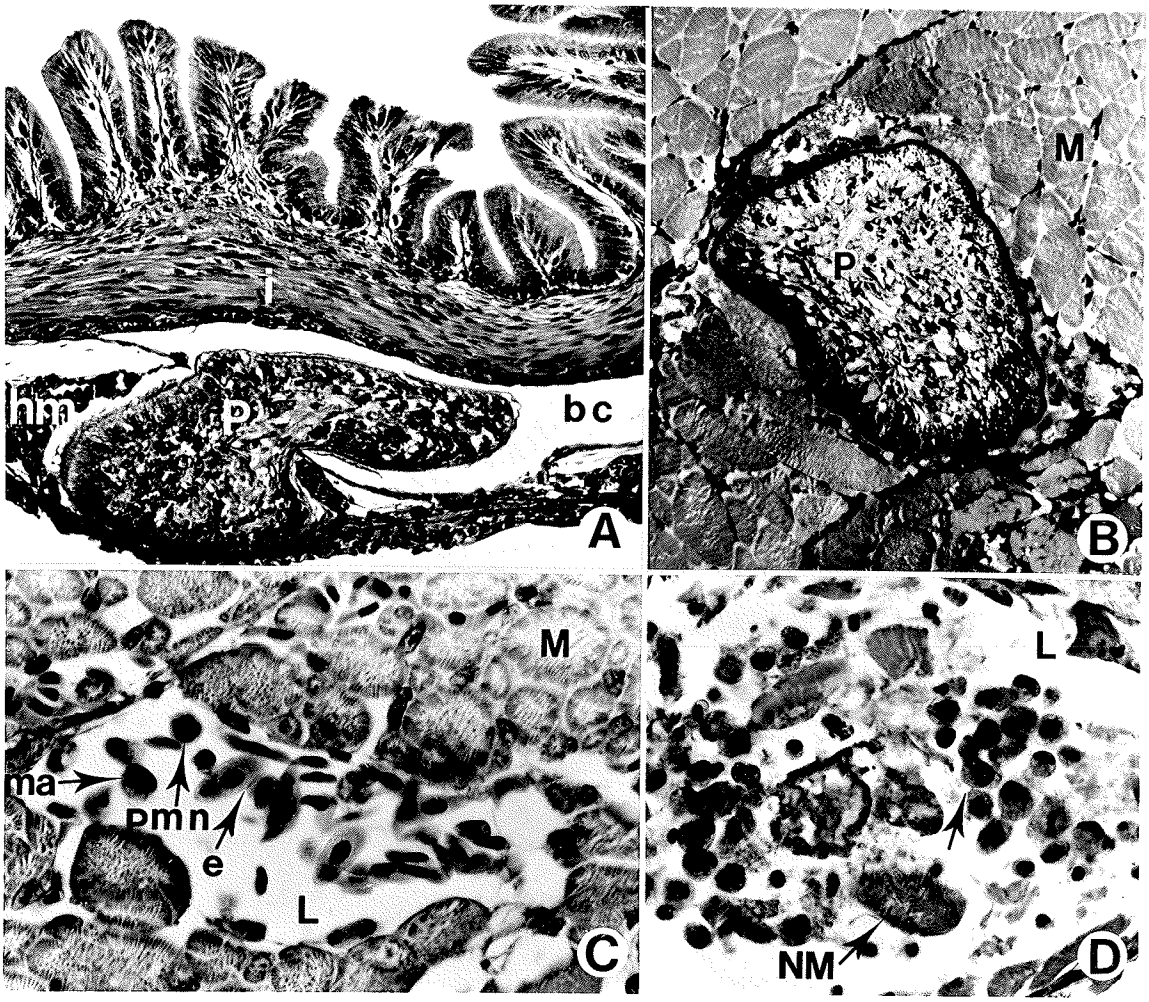

A
V
P
P
C
pc
P
D
pc
B
P
bc
D

FIG. 6. Migration of plerocercoids of *T. crassus* in the muscle of whitefish at 17, 22, 30, 40 and 63 Days PI as reconstructed from serial sections. (17) Plerocercoids occasionally entered lesions made by another worm and the scoleces were away from the main lesion. (22) Scoleces of plerocercoids have turned back into main lesion. (30) Plerocercoid entered muscle from posterior body body cavity at Δ , migrated posteriorly and then migrated anteriorly through the same lesion as depicted by arrows. (40) Plerocercoid reentered body cavity from muscle, moved anteriorly and reentered muscle at Δ as depicted by arrows. (63) Plerocercoid wound throughout musculature and broke through integument from ventral hypaxial muscle. Note non-uniformity of capsule formation.

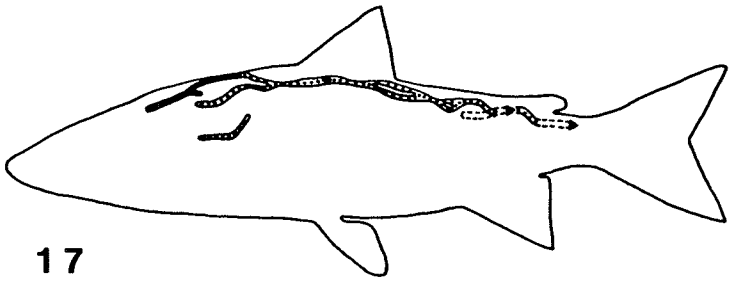

bc
P
A
M
P
B
M
ma
pmn
e
L
C
L
NM
D

(Fig. 5B) at which time they were usually visible under the integument of live whitefish. Migration of worms across myosepta and myotomes produced muscle necrosis and a chronic inflammatory response. This response was initially characterized by the formation of lesions which were considered to be pathological or traumatic discontinuity in the muscle caused by the migrating worm. Lesions were present around the plerocercoid and in muscle through which the worm had migrated. Hemorrhaging into lesions vacated by the plerocercoid (=LVP) during its migration was evident between Days 17-30 PI (Fig. 5C), and was the first clinical sign of infection in live whitefish. Infiltration of macrophages (=histiocytes) and polymorphonuclear (pmn) leucocytes (Appendix VIII) into lesions (LVP) was evident by Day 30 PI (Fig. 5D), but not around plerocercoids up to Day 50 PI (Figs. 2A, 2B, 2C and 2D).

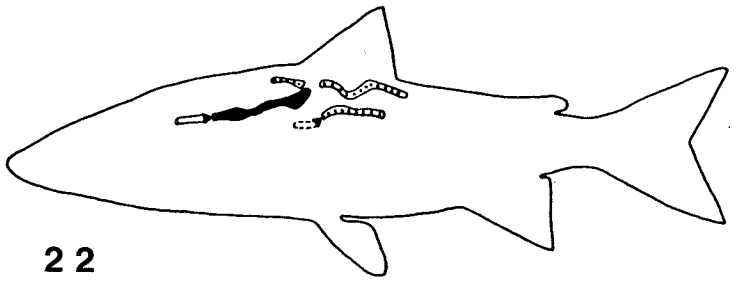
It was possible to reconstruct the migration of plerocercoids during the first 40 days of infection by tracing lesions through the muscle of whitefish from serial sections. The early migration of plerocercoids often covered considerable distances in the muscle (Fig. 6, Day 17 PI). Plerocercoids looped back on themselves (Fig. 6, Days 22 and 30 PI), entered established lesions (LVP) (Fig. 6, Day 17 PI) and left the muscle, reentered the body cavity and then reinvaded the muscle (Fig. 6, Day 40 PI).

After Day 40 PI, worms wound throughout the myotomes (Figs. 6, Day 63 PI and 7A), and sometimes replaced the entire muscle field in a given region (Fig. 7B). The anterior, middle and posterior regions of plerocercoids occassionally penetrated into the body cavity (Fig. 7C, Table 1) or through the integument (Fig. 7D, Table 1). In the latter case there was marked hyperplasia of the epithelium (Fig. 7D).

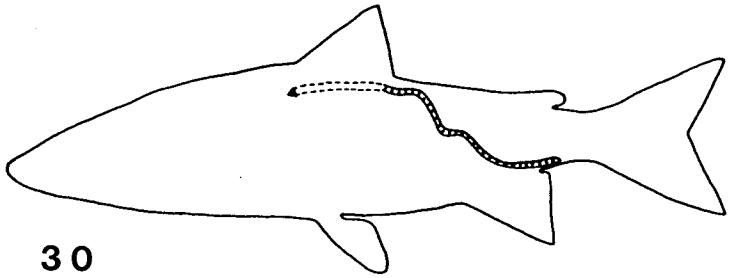
FIG. 6. Migration of plerocercoids of *T. crassus* in the muscle of whitefish at 17, 22, 30, 40 and 63 Days PI as reconstructed from serial sections. (17) Plerocercoids occasionally entered lesions made by another worm and the scoleces were away from the main lesion. (22) Scoleces of plerocercoids have turned back into main lesion. (30) Plerocercoid entered muscle from posterior body body cavity at Δ , migrated posteriorly and then migrated anteriorly through the same lesion as depicted by arrows. (40) Plerocercoid reentered body cavity from muscle, moved anteriorly and reentered muscle at Δ as depicted by arrows. (63) Plerocercoid wound throughout musculature and broke through integument from ventral hypaxial muscle. Note non-uniformity of capsule formation.



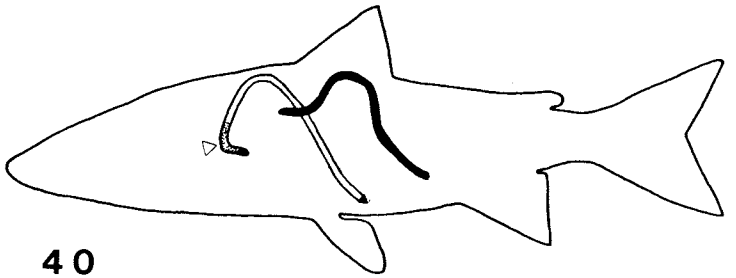
17



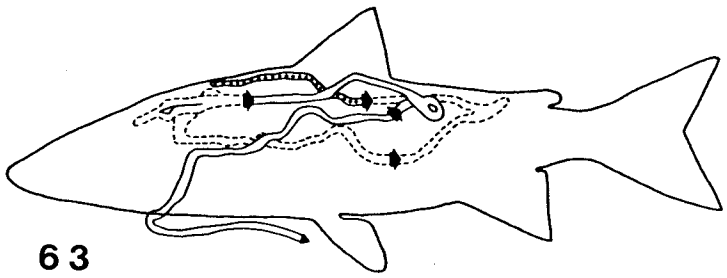
22



30



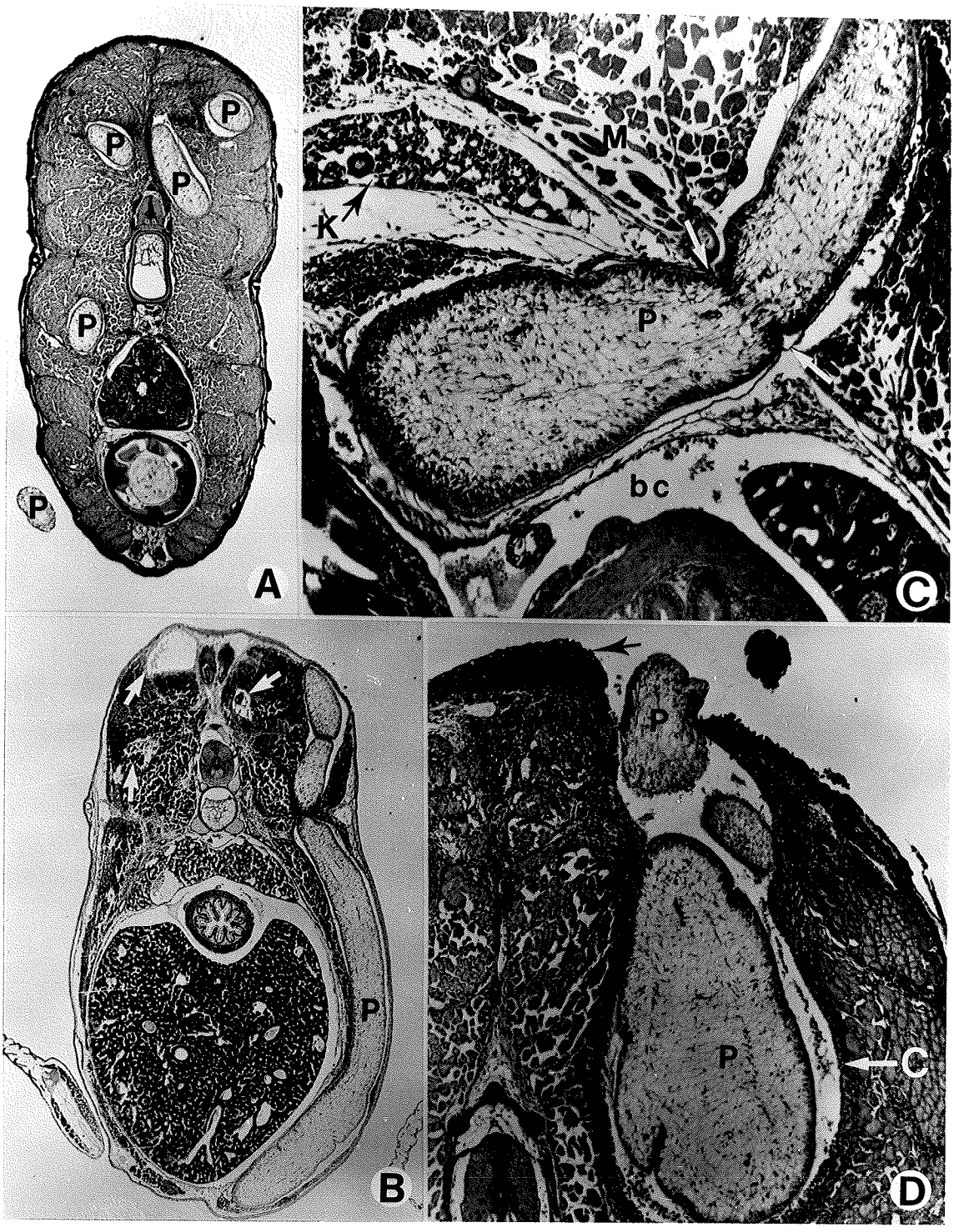
40



63

- WORM LEFT
- WORM RIGHT
- WORM BODY CAVITY
- LESION LEFT
- LESION RIGHT
- SCOLEX
- CAPSULE FORMATION

FIG. 7. (A) Section through region of rectum at Day 63 PI. This fish had one plerocercoid which wound extensively in the muscle and broke through the integument. H & E, X 20. (B) Section through region of liver at Day 60 PI. Note vacated lesions (arrows) in muscle and almost total replacement of hypaxial muscle on one side by plerocercoid. Mallory's trichrome, X 20. (C) Plerocercoid in body cavity and muscle at Day 40 PI. Note constriction in worm (arrows) where it broke into body cavity. H & E, X 120. (D) Plerocercoid breaking out of capsule and through integument at Day 90 PI. Note hyperplasia (arrow) of epithelium. H & E, X 70. bc=body cavity, C=host capsule, K=kidney, M=muscle and P=plerocercoid.


P
P
P
P
P
A
M
K
P
bc
C
P
B
P
P
C
D

Capsule and granuloma formation

Coincident with coiling of plerocercoids after Day 60 PI was the infiltration of macrophages, pmn leucocytes and fibroblasts into the lesion surrounding the worm which indicated capsule formation. Proliferation of fibroblasts around the outer perimeter of lesions (LVP) at this time signalled granuloma formation, and hemorrhaging into these lesions subsided. The term granuloma as used in this study describes a tumor-like mass or nodule of granulomatous tissue which does not enclose the infectious agent (i.e. plerocercoid). Capsule is defined as the host reaction around the plerocercoid and is not to be confused with a cyst which is a response of parasite origin. Granulomas found in the muscle (Fig. 8A), body cavity (Fig. 8B) and kidney included a central core of necrotic muscle interspersed with macrophages and pmn leucocytes surrounded by concentric whorls of fibroblasts. One fish, in which the plerocercoid broke out of the capsule and through the integument at Day 70 PI, had a granuloma just under the integument by Day 110 PI (Fig. 8C, see also Fig. 11, Day 110 PI). Granulomas often extended along most of the length of a fish (Fig. 8D).

The cells involved in capsule formation were the same as those in granulomas. These cells accumulated in a non-uniform pattern along the length of the plerocercoid at the beginning of capsule formation (Figs. 6, Day 63 PI and 9A). Capsules were fully formed around plerocercoids by Day 70 PI. The capsule wall in muscle and the body cavity was composed of an outer layer of fibroblasts and an inner layer of degenerating macrophages and pmn leucocytes, both of which varied in thickness (Fig. 9B). Only the fibroblast layer was present in some regions of the capsule (Fig. 9C). Collagen desposition by fibroblasts

FIG. 8. (A) Granuloma in epaxial muscle at Day 110 PI. Central core of degenerating macrophages and leucocytes is surrounded by fibroblasts. H & E, X 160. (B) Granuloma in body cavity at Day 100 PI. H & E, X 60. (C) Granulomas (arrows) in epaxial muscle at Day 110 PI. One granuloma is just under the integument where a plerocercoid escaped. Note hyperplasia of epithelium. H & E, X 20. (D) Longitudinal section through whitefish at Day 66 PI. Extensive granuloma (arrows) and plerocercoid are in epaxial muscle. H & E, X 10. a=anus, bc=body cavity, F=fibroblasts, G=granuloma, hy=hyperplasia of epithelium, Le=leucocytes, M=muscle and P=plerocercoid.

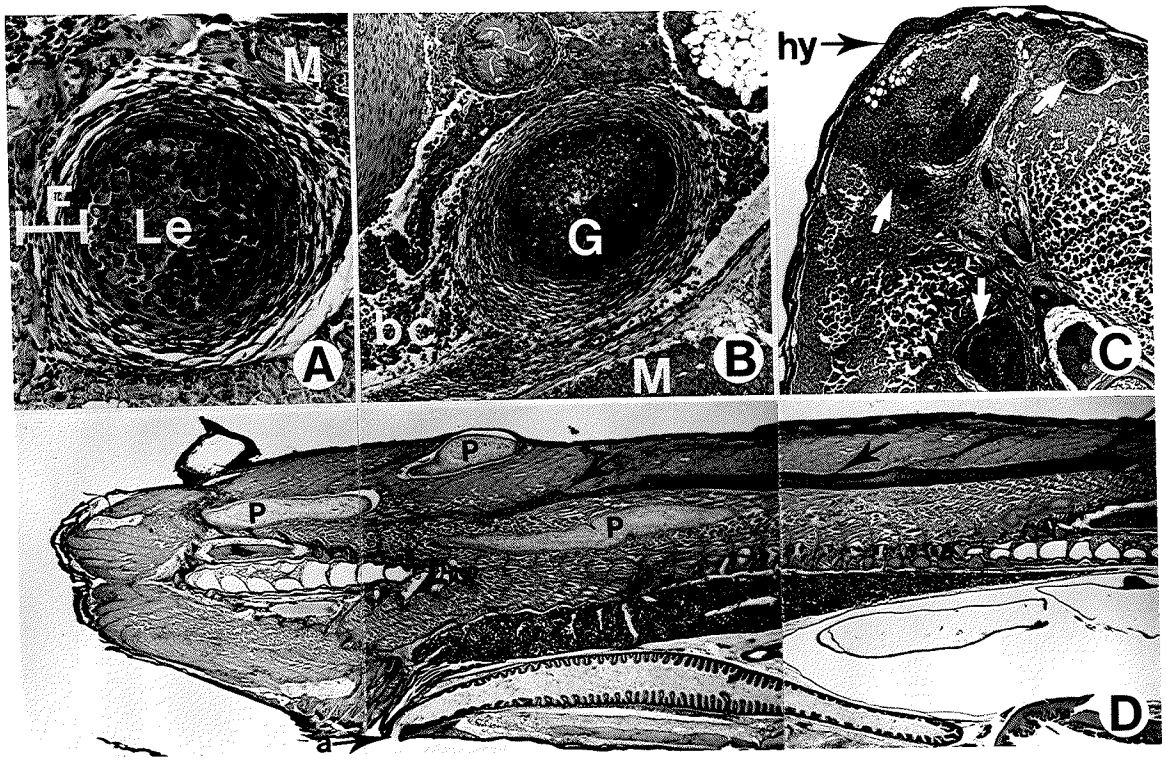
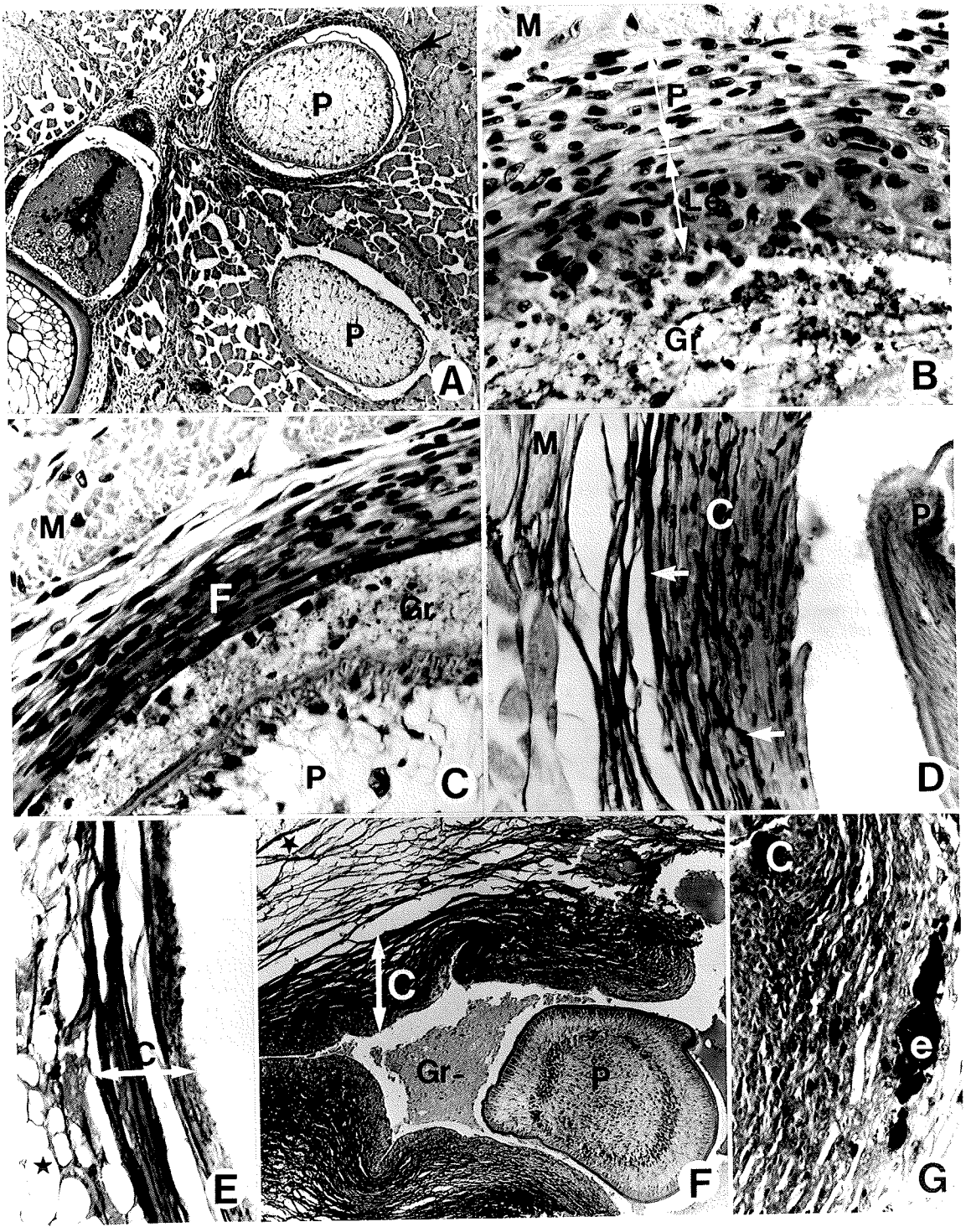

M
Le
A
G
bc
M
B
hy
C
P
P
P
a
D

FIG. 9. (A) Sections through a plerocercoid in epaxial muscle at Day 63 PI. Note accumulation of inflammatory cells (arrow) around upper section of plerocercoid and almost total absence of reaction around lower section of the same plerocercoid. H & E, X 80. (B) Host capsule in epaxial muscle at Day 80 PI. Capsule is comprised of an inner layer of degenerating leucocytes and macrophages and an outer layer of fibroblasts. Note degenerating cells and granular material within capsule. H & E, X 540. (C) A different region of the same host capsule as in B. Capsule wall around plerocercoid is composed of the outer layer of fibroblasts only. H & E, X 500. (D) Collagen (arrows) in capsule wall surrounding plerocercoid in epaxial muscle at Day 100 PI. Picro-sirius red, X 500. (E) Degenerating capsule from hypaxial muscle at 2.5 years PI. Note reduced capsule wall and diffuse, non-cellular tissue (star) around capsule. Picro-sirius red, X 110. (F) Capsule containing live plerocercoid at 2.5 years PI from same fish as capsule in E. Note thickness of capsule wall and associated non-cellular tissue (star). Picro-sirius red, X 80. (G) Capsule in F showed evidence of vascularization. Mallory's trichrome, X 260. C=host capsule, e=erythrocytes, F=fibroblasts, Gr=granular material, Le=leucocytes, M=muscle and P-plerocercoid.


P
P
A
M
P
Le
Gr
B
M
F
Gr
P
C
M
C
P
D
C
E
C
Gr
P
F
C
e
G

in the outer layer of capsule walls and granulomas was detectable at Day 70 PI and obvious by Day 100 PI (Fig. 9D). Walls of capsules lacking plerocercoids were thinner (Fig. 9E) and contained less collagen than capsules containing live worms (Fig. 9F) at 2.5 years PI. A network of blood vessels was present in the walls of capsules with live worms at this time (Fig. 9G). Both types of capsules were surrounded by a diffuse, non-cellular tissue (Figs. 9E and 9F).

A dense, granular, PAS-positive material containing degenerating cells was present in the lumen of the capsule along with plerocercoids by Day 80 PI (Figs. 9Band 9C). Capsules examined from experimentally infected fish at 1.5 - 3 years PI and recovered from naturally infected fish had plerocercoids positioned around the periphery of a compact core of this material, which contained small calcium deposits (Fig. 10A). The hooks of one plerocercoid were observed to be embedded in the capsule wall at Day 90 PI, while the anterior region of another plerocercoid was not surrounded by a host capsule in the hypaxial muscle of a fish at 1.5 years PI.

Capsules had various shapes, were continuous at one or more points with granulomas and not restricted to a specific area of the fish (Fig. 11, Days 70, 80 and 90 PI). Capsules in the body cavity (Fig. 10B) were not continuous with granulomas, but granulomas in muscles marked the point of reentry of the plerocercoid into the body cavity (Fig. 11, Day 120 PI). Capsules replaced extensive areas of fish muscle (Fig. 11, Days 70, 80 and 90 PI), and caused regional changes in fish shape (Figs. 10C, 10D and 10E).

FIG. 10. (A) Encapsulated plerocercoid in epaxial muscle from natural infection. Note plerocercoid arranged around periphery of central core of granular material within capsule. H & E, X 20. (B) Section through encapsulated plerocercoid in body cavity at Day 120 PI. H & E, X 40. (C) Section through region of rectum at Day 80 PI. Encapsulated plerocercoid (arrow) occupies almost entire field of epaxial muscle. H & E, X 20. (D) Section through region posterior to anus at Day 90 PI. Encapsulated plerocercoid (arrow) occupies most of epaxial and hypaxial muscle on one side. H & E, X 20. (E) Section through region of stomach and small intestine at Day 100 PI. Note encapsulated plerocercoid (arrow) is distributed throughout muscle on one side of fish. H & E, X 10. bc=body cavity, C=host capsule, Gr=granular material, M=muscle and P=plerocercoid.

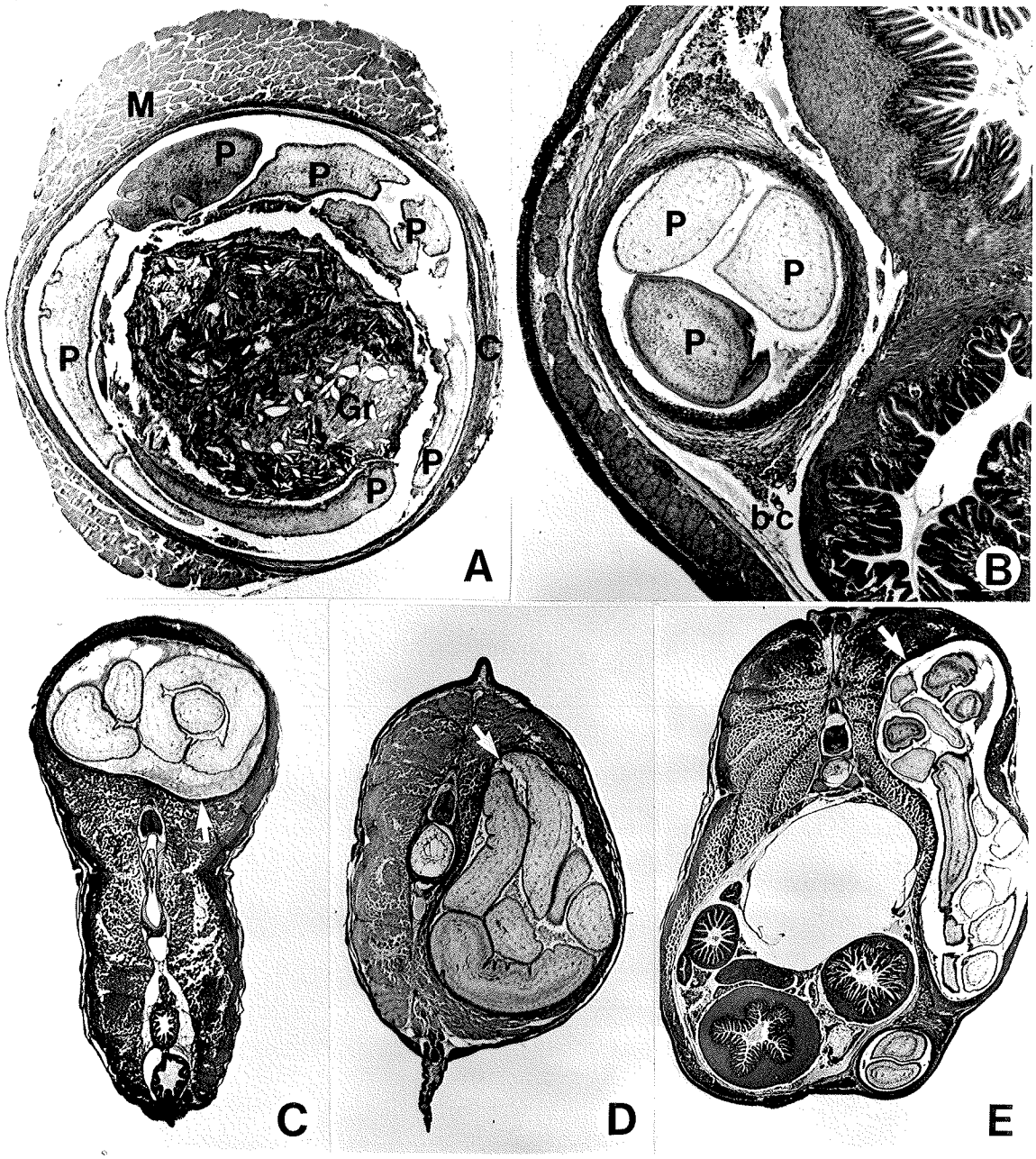
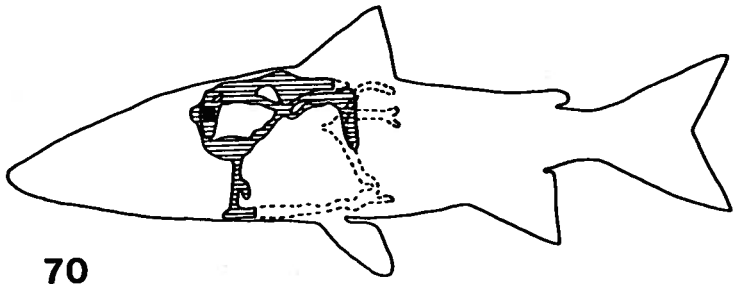
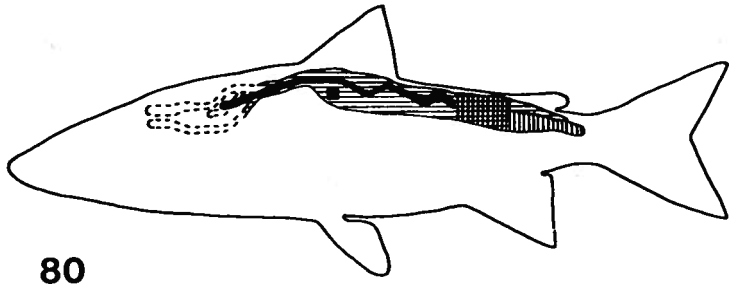

M
P
P
P
P
P
C
Gr
P
P
P
A
P
P
P
bc
B
C
D
E

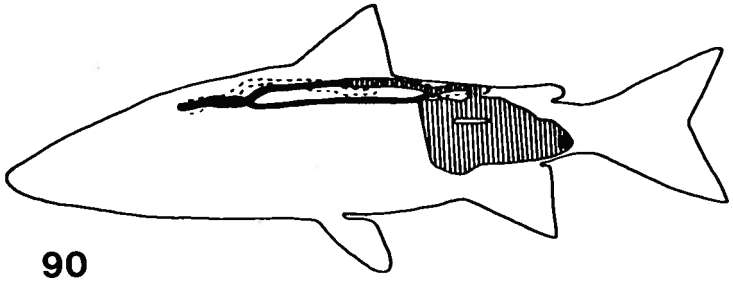
FIG. 11. Encapsulation of plerocercoids of *T. crassus* in the muscle and body cavity of whitefish at 70, 80, 90 and 120 Days PI as reconstructed from serial sections. (70, 80 and 90) Note granulomas continuous with host capsules. (110) Plerocercoid broke through integument and out of the fish leaving a network of granulomas in the epaxial muscle. (120) Plerocercoid probably entered body cavity from muscle at Δ and became encapsulated.



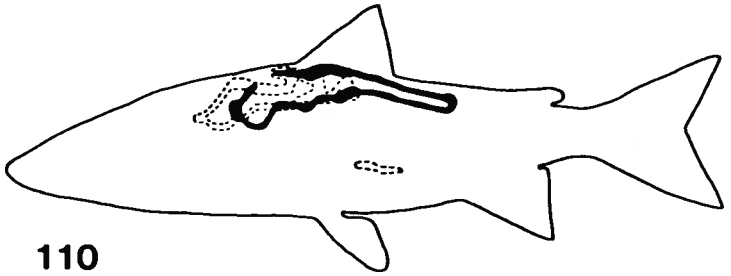
70



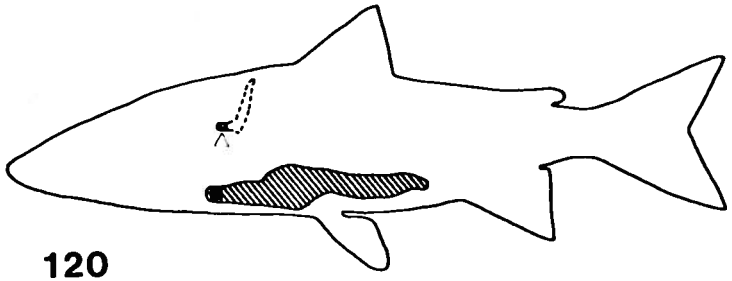
80



90



110



120

CAPSULE LEFT

CAPSULE RIGHT

CAPSULE BODY CAVITY

GRANULOMA LEFT

GRANULOMA RIGHT

GRANULOMA BODY CAVITY

SCOLEX

Experimental transplants

All plerocercoids recovered from the transplants were dead. Worms were stretched along the length of the body cavity or entwined around viscera. A small amount of connective tissue was present around plerocercoids by Day 21 PI. Most plerocercoids were completely surrounded by connective tissue by Day 28 PI, and all were in advanced stages of degeneration. The host response to plerocercoids was most intense around worms which were wrapped around the intestine.

General effects on host

Mortality of infected whitefish was 21.7% (10/46), and 70% of this mortality occurred between Days 48-59 PI. Dead fish were often found with plerocercoids penetrating through their integument. There was no mortality of infected fish prior to Day 19 PI or after Day 60 PI, and none of the control fish died. Distortion of body shape during and following encapsulation impaired the swimming of infected whitefish (Fig. 10C, 10D and 10E). These changes in swimming behavior included decreased activity and loss of bouyancy. No significant differences were found between the fork lengths of infected and control fish at Days 30, 55 and 80 PI (Fig. 12). Glycogen levels in the livers of infected fish did not differ from uninfected fish at Days 30, 55 and 80 PI (Table 2).

FIG. 12. Fork lengths ($\bar{X}$ ± S.D.) of infected and control whitefish at four time intervals. (* number of fish).

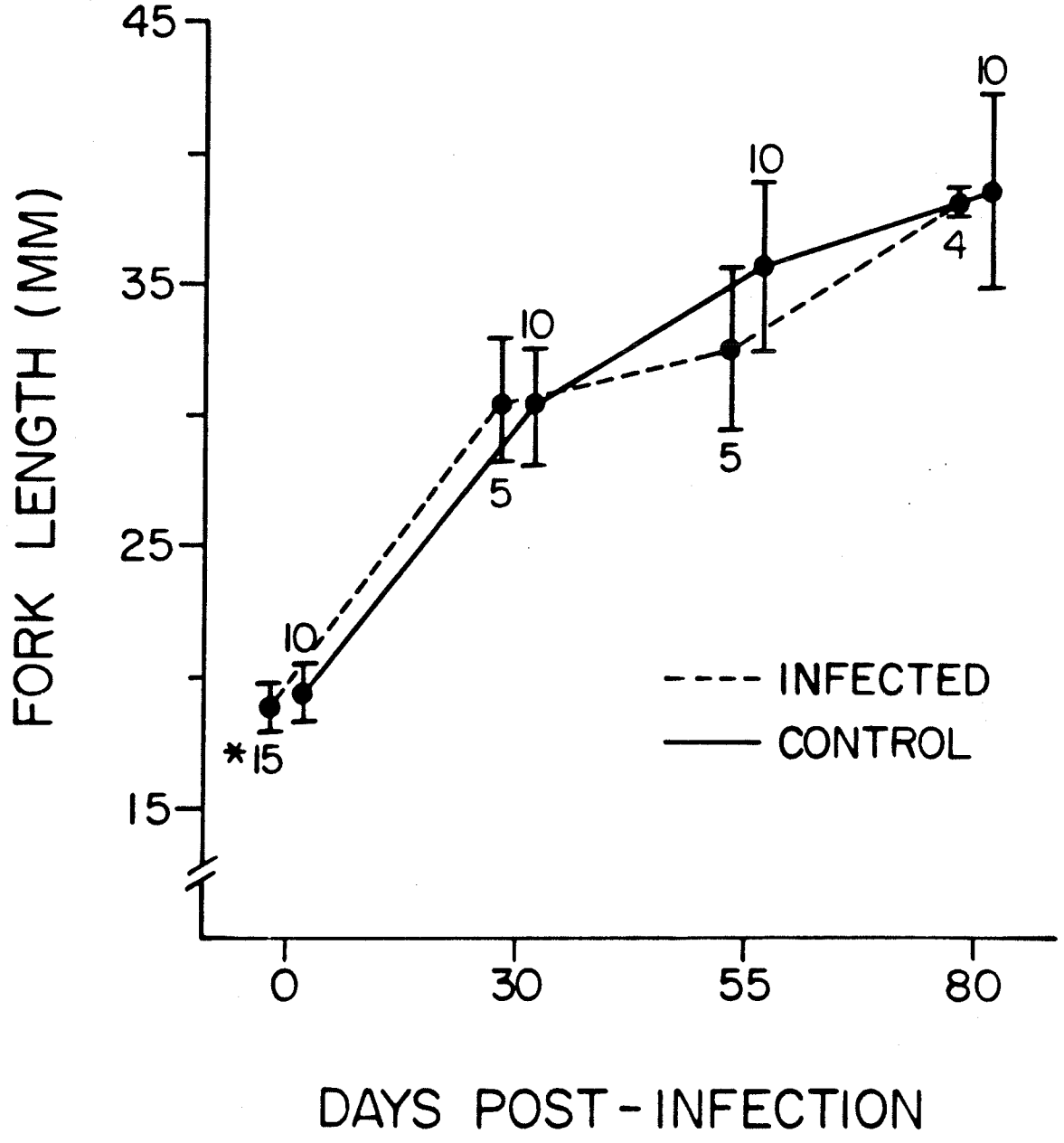

45
35
25
15
FORK LENGTH (MM)
10
10
10
10
5
5
4
*15
INFECTED
CONTROL
0
30
55
80
DAYS POST-INFECTION

TABLE 2. Liver glycogen in whitefish infected with the plerocercoid of *T. crassus* (=I) and in control (=uninfected) fish (=C). [+ = low glycogen, ++++++ = high glycogen].

FISH	30 @	55	80
I*	++++	++++++	+++++
C*	+++	++++++	+++++

* whitefish were one month in age at start of experiment.

@ day post-infection.

Discussion

Plerocercoid growth, differentiation and movement

Prior to this study, growth of T. crassus plerocercoids was reported to occur in the body cavity, with the plerocercoids entering the flesh just before encapsulation (Miller 1952). This study clearly showed that plerocercoids entered the muscle as early as Day 5 PI and the majority of growth occurred there. The rapid growth rate of T. crassus from Days 1-60 PI was similar to that found for plerocercoids of S. solidus (Muller) (Orr and Hopkins 1969). The variability in rates of growth of individual plerocercoids was probably related to the condition of the original procercoids at the time of infection, intraspecific competition between plerocercoids and genetic variation within the species. These factors may also be responsible for the range in sizes observed in older plerocercoids.

Hooks were observed in T. crassus by Days 80-100 PI in whitefish at 15-17^{o}C, but this time for hook formation was considerably longer than the times noted by Kuperman (1973): T. nodulosus (Pallas) in perch (40 days), T. amuriensis Kuperman in goldfish (42 days) and T. orientalis Kuperman in the Amur sleeper (39 days). These differences may relate to differences in temperature of experiment, species of Triaenophorus or its hosts. Perhaps the hypertrophied tegument was a site of protein synthesis for hook formation in T. crassus as suggested for Taenia crassiceps (Zeder) (Mount 1970). Collagenous tissue on the outer edge of both developing and formed hooks probably represented muscle attachment sites.

The only information, to our knowledge, on the movements of developing plerocercoids is a few cursory comments on Spirometra mansonoides Mueller by Mueller (1959b). The sequence of movements observed

for developing plerocercoids of T. crassus in vitro was assumed to be similar to that in tissue, and certainly would be conducive to movement through tissue. The phallus-like expansion of the anterior end of the plerocercoid followed by formation of a bulge at the mid-body of the worm would allow it to anchor itself in the lesion prior to thrusting forward into new tissue. The distance/unit time travelled in tissue would probably be less than in vitro (i.e., 2.5 mm/minute) due to the resistance of tissue and time required to break down muscle and connective tissue. This type of movement may be common for plerocercoids moving through tissues.

Plerocercoid migration

This study provided new insights on the migration of plerocercoids in muscle. Developing plerocercoids revealed a variety of migration patterns and appeared to follow a path of least resistance in the muscle by orienting parallel to muscle fibers prior to Day 30 PI. A decrease in plerocercoid activity coupled with a dramatic increase in worm size after Day 30 PI suggested that new muscle occupied by worms after this time was more attributable to plerocercoid growth rather than active migration. This significant increase in plerocercoid size resulted in winding of the plerocercoid through the muscle and penetration of the worm into the body cavity or through the integument.

Capsule and granuloma formation

The time required for capsule formation in these experimental infections was similar to the two month period estimated for natural infections (Miller 1952), and indicated that the time frame for the progression of plerocercoid growth, migration,differentiation and host pathology was similar in this

study and natural infections. Encapsulation of T. crassus in whitefish was much slower than encapsulation of other pathogens and repair of damaged tissue by fish (Lee and Cheng 1970; Finn and Nielson 1971; Roberts et al. 1973; Sommerville 1981). The delayed encapsulation of T. crassus may be related to hook formation which occurred simultaneously with capsule formation. Antigenic material associated with hook formation may have elicited the host cellular response observed around the plerocercoid. Notably, encapsulated plerocercoids of T. crassus without hooks were not found in natural infections of cisco and whitefish. Other aspects of plerocercoid differentiation, such as the disappearance of the subtegumental secretory glands and secretory projections of the tegument (Kuperman and Davydov 1981), may be associated with encapsulation.

Capsule formation began at the end of the rapid growth phase of the plerocercoid. A non-uniform host response at the beginning of capsule formation has not been observed for other helminths in fish muscle. This variable host response was probably associated with the amount of damage in a particular area of the fish and the location of the worm relative to the perimysium, a probable source of fibrogranulation tissue in fish (Finn and Nielson 1971; Roberts et al. 1973). The result of plerocercoids contracting and coiling on themselves after Day 60 PI was a granuloma continuous with the host capsule. Uniform capsules occurred when plerocercoids coiled in a compact manner in the muscle or were present in the body cavity. Less pronounced coiling in the muscle produced diffuse capsules. It is likely that variation in plerocercoid coiling produces the various capsule shapes reported from the muscle of fish naturally infected with T. crassus (Hjortland 1928; Miller 1945b). Plerocercoids breaking out of capsules also influenced capsule shape, but this was a rare event in our experimental infections.

Miller (1952) suggested that plerocercoids of T. crassus lived 4-5 years in whitefish and cisco, and based this interpretation on the morphology of the host capsule and infection intensities in year classes of fish. Use of capsule morphology to determine age of infection is questionable as capsules containing living worms and capsules without worms were recovered from a fish at 2.5 years PI. It is clear that degeneration of some worms occurred prior to the 4-5 year period proposed by Miller (1952), although this study showed that some plerocercoids can live at least 3 years.

Experimental transplants

Fully differentiated plerocercoids of T. crassus occassionally broke out of the host capsule in experimental infections of whitefish fry. The experimental transplants clearly showed that fully differentiated worms reentering the body cavity will not survive. All plerocercoids in the body cavity in experimental infections of whitefish fry were under 70 days in age with one exception, and had reentered the body cavity from the muscle prior to the completion of their development and encapsulation by the host. The single fully differentiated plerocercoid in the body cavity at Day 120 PI had previously disappeared from the muscle of a whitefish fry between Days 60-70 PI. Thus, plerocercoids of T. crassus evidently possess the capacity to survive in the body cavity only if they reenter this site from the muscle prior to the completion of their development and encapsulation by the host. Plerocercoids in the body cavity were rare in experimental infections of whitefish fry (Table 1) and in naturally infected whitefish (T. Dick, pers. comm.) and probably play an insignificant role in the transmission of T. crassus in natural systems.

General effects on host

Mortality of infected whitefish fry in this study was considerably less (i.e., 21.7%) than the 54.5% reported by Dick and Rosen (1982), but can be explained by differences in experimental design. Some infected fish which might have died during the present experiments were necropsied or fixed for histological examination, thus reducing the level of mortality. The maximum period of host mortality occurred between Days 48-59 PI corroborating the findings of Dick and Rosen (1982). The large size of the plerocercoid and its extensive winding through the muscle at this time clearly contributed to this mortality. Rupturing of the integument by plerocercoids may have been associated with host death and/or a post-mortem response of plerocercoids in some dead fish. There was evidence of healed wounds in fish which had lost plerocercoids. It is likely that many 0 age whitefish naturally infected with *T. crassus* also die, as both experimental fish in this study and wild fish were of a similar size and infection intensities were similar (Watson 1977). Furthermore, the impaired swimming behavior of fish infected with *T. crassus* may increase mortality in the field by fascilitating predation as shown by Rogers *et al.* (1972).

A decrease in liver glycogen due to uptake of glucose by plerocercoids or stress associated with the infection, was not observed in whitefish fry infected with *T. crassus*. Depletion of liver glycogen is a secondary alteration of the general adaptive syndrome (GAS) in fish under chronic stress (Wedemeyer and McLeay 1981), and it was surprising that this did not occur in light of the high mortality observed. Similarly, impaired growth, a tertiary effect of the GAS, was not observed in

infected whitefish. Perhaps whitefish fry adapt rapidly to infection with T. crassus by increased food intake or decreased activity.

The high host mortality resulting from plerocercoid migration initially appears deleterious to the transmission of T. crassus. However, the long migration phase and slow differentiation of plerocercoids may be beneficial since they allow the worm to increase its size and energy reserves, and thus its life span. This life span of plerocercoids is important in the transmission of T. crassus. Coracidia of T. crassus are present for a brief period each year, and if the susceptible stages of the first intermediate host are not available, then there will be little or no recruitment of parasites in that year. The extended life span of T. crassus plerocercoids provides a reserve of potential adult worms and their coracidia in the event that infection of the first intermediate host does not occur in a particular year.

CHAPTER III

EXPERIMENTAL INFECTIONS OF RAINBOW TROUT, *SALMO GAIRDNERI*, WITH PLEROCERCOIDS OF *TRIAENOPHORUS CRASSUS*

Introduction

Triaenophorus crassus occurs as an adult in the small intestine of the northern pike and as a plerocercoid in the flesh of a variety of salmonid fish (Kuperman 1973). There is little information on mortality of fish naturallly infected with this parasite (Michajlow 1962), but recent studies have shown that T. crassus plerocercoids killed experimentally infected whitefish fry (Dick and Rosen 1982; see Chapter 2). Interestingly, T. crassus has not been reported from rainbow trout, Salmo gairdneri, in Canada (Margolis and Author 1979), but is included as a a parasite of Salmo irideus by Kuperman (1973). The objectives of this study were to determine the suitability of S. gairdneri as a host for T. crassus in North America, and to assess the pathology and mortality of this experimental host.

Materials and methods

Gravid adults of T. crassus were obtained from spawning northern pike at Falcon Lake, Manitoba (95° 20'W, 49° 40'N) during the spring of 1982. Cyclops bicuspidatus thomasi were collected from ponds in the vicinity of Winnipeg, Manitoba, and six-month old rainbow trout (group-domestic Nisqually) were donated by the Rockwood Experimental Fish Hatchery, Manitoba. The methods for processing adult tapeworms, culturing coracidia, infecting C. b. thomasi and maintenance of fish have been described in Chapter 1. Trout were divided into two groups of 25 fish each and acclimated to a water temperature of 15-17°C for five days. One group of fish was exposed to large numbers of infected cyclopids following the methods in Chapter 1 while the other group was exposed to Artemia only. Fish were held at 15-17°C following exposure to infected cyclopids or Artemia and fed equal volumes of Artemia daily which were adjusted upon death or removal of infected fish.

Infected fish were sacrificed at selected times (i.e., 30, 45, 49, 58, 59 and 75 Days post-infection (PI)), fixed whole in Bouin's, embedded in paraffin and serially sectioned at 10 uM with a rotary microtome. Slides were stained with hematoxylin and eosin, PAS-hematoxylin (Humason 1979) and picro-sirius red (Puchtler et al. 1973). Infected fish which died during the experiment were necropsied and plerocercoids recovered were fixed, stained, mounted and their lengths determined.

Results

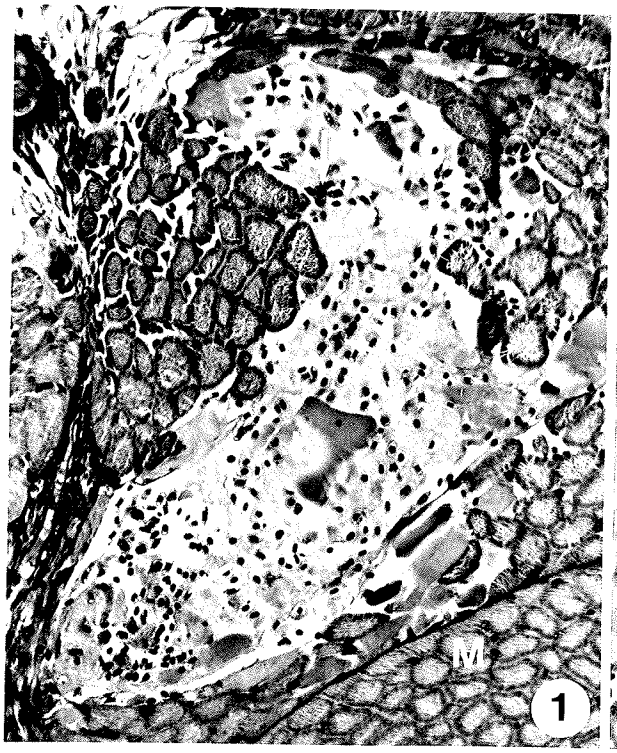
Positive infections were obtained in 11/25 of the exposed trout and the mean intensity of infection was 1.7 (range 1-6). Hemorrhaging into lesions (Fig. 1) created by migrating plerocercoids in the muscle was the first clinical evidence of parasitism between Days 22-58 PI ($\bar{X}$ = 37 Days PI). Lesions were considered to be any pathological or traumatic discontinuity in the muscle. Worms were found in a variety of sites within trout (Table 1). Plerocercoids were observed in the muscle of sectioned trout by Day 30 PI (Fig. 2), but extensive vacated lesions in the muscle at this time indicated that worms entered this tissue much earlier. Worms caused muscle necrosis (Fig. 1), and replaced considerable areas of muscle by Day 56 PI, having attained an average length of 45.8 ± 28.6 mm (N = 13) between Days 44-56 PI. Plerocercoids were wound throughout the myotomes after Day 44 PI (Fig. 3) and often penetrated into the body cavity (Fig. 4). A loss of pigmentation on one side of a trout was noted in which the plerocercoid had penetrated through the integument and into the vertebral cavity (Fig. 5), brain (Fig. 6) and gill chamber (Fig. 7). Infiltration of macrophages and polymorphonuclear leucocytes into vacated lesions (Fig. 8) led to formation of granulomas between Days 45-75 PI. Granulomas were tumor-like masses or nodules of granulomatous material which did not enclose the plerocercoid, and were comprised of a central core of necrotic cell material surrounded by fibroblasts (Fig. 9). Plerocercoids were still wound through the musculature and were not enclosed in a host capsule by Day 75 PI (Fig. 3). The initiation of hook development was observed in the scolex region by Day 75 PI (Fig. 10).

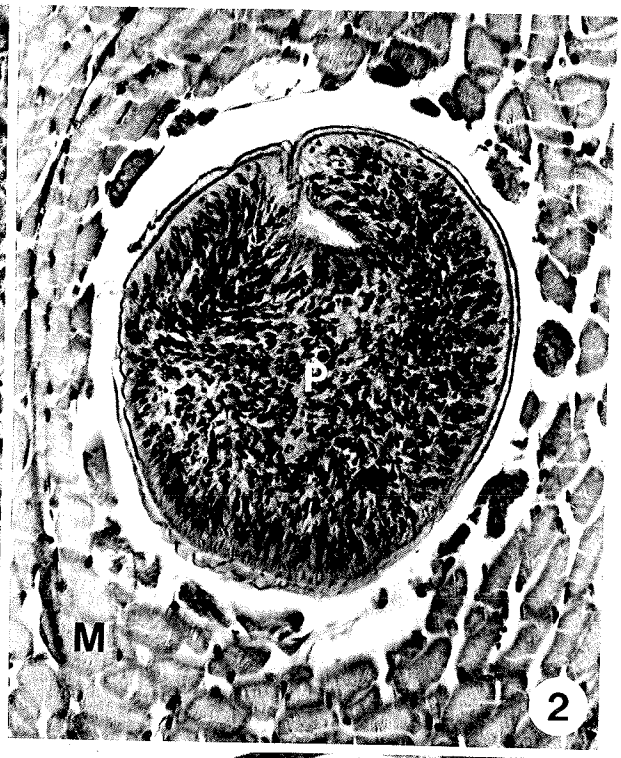
FIG. 1. Lesion (L) in muscle (M) at Day 45 PI. Note extensive hemorrhaging (arrows) into lesion; hematoxylin and eosin (H & E), X 330.

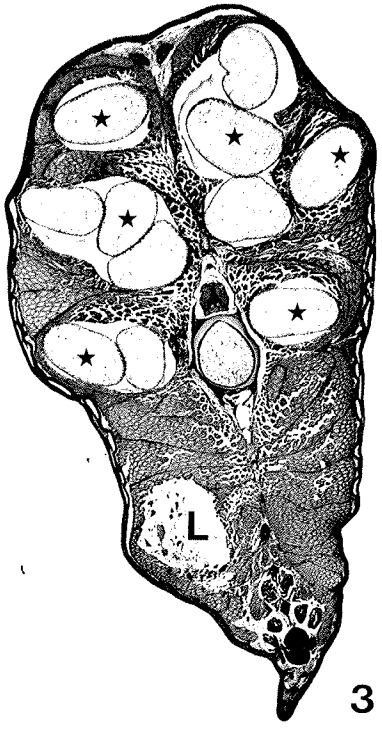
FIG. 2. Plerocercoid (P) in muscle (M) at Day 30 PI; H & E, X 280.

FIG. 3. Single plerocercoid (stars) wound throughout muscle in post-anal region of trout at Day 75 PI. Note lesion (L) in hypaxial muscle; H & E, X 20.

FIG. 4. Plerocercoid (P) reentering body cavity (B) from muscle (M) at Day 45 PI: I = intestine and S = swim bladder; H & E, X 70.


M
1


P
M
2


L
3

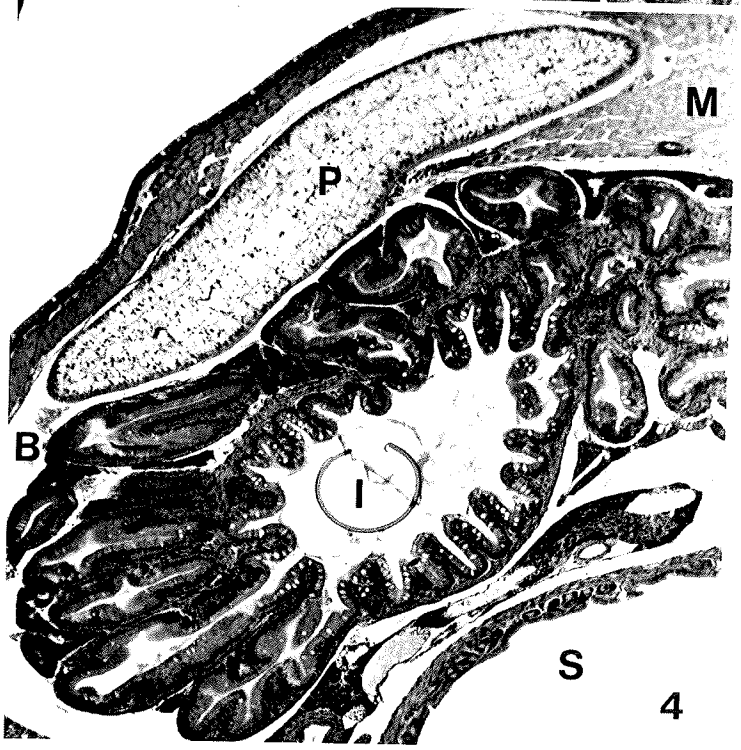

M
P
B
I
S
4

TABLE 1. Location of 19 plerocercoids of T. crassus in experimentally infected rainbow trout held at 15-17°C.

DAYS PI	N*	Muscle	Muscle‡	Muscle & Body Cavity	Muscle ‡ & Body Cavity	Body Cavity	Muscle‡ & Vertebral Cavity & Brain
30-45	10	8	0	1	0	1	0
46-55	4	0	2	2	0	0	0
56-75	5	1	1	1	1	0	1

* N = number of plerocercoids.
‡ Plerocercoid penetration through integument.

FIG. 5. Sections through a single plerocercoid (P) at Day 58 PI. Note worm in vertebral cavity (arrow), muscle (M) and breaking through host integument; H & E, X 40.

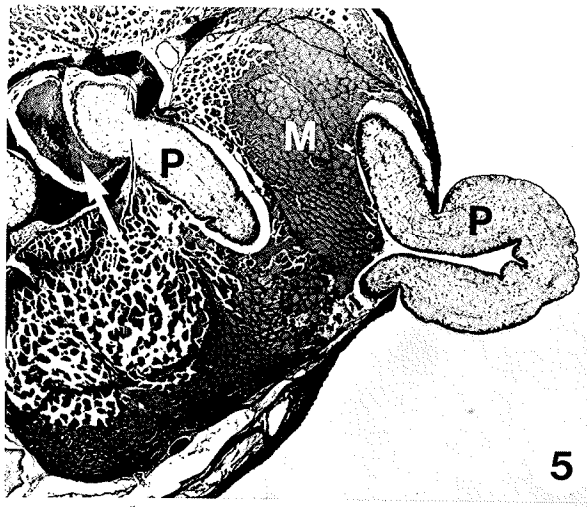
FIG. 6. Same plerocercoid (P) as in Fig. 5 penetrating into brain (BR). Note lesion in brain (arrow) and extensive hemorrhaging (H) adjacent to left eye; PAS-hematoxylin, X 50.

FIG. 7. Same plerocercoid (P) as in Figs. 5 and 6 penetrating into gill chamber (GC) from muscle (arrows). Note hemorrhaging (H) associated with worm in muscle; PAS-hematoxylin, X 50.

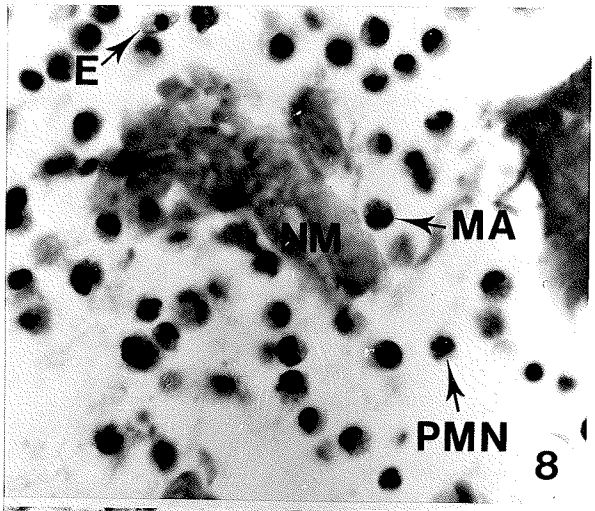
FIG. 8. Macrophages (MA), polymorphonuclear leucocytes (PMN) and erythrocytes (E) in muscle lesion shown in Fig. 3. Note necrotic muscle (NM); H & E, X 820.

FIG. 9. Granuloma (G) in muscle (M) at Day 45 PI: H & E, X 610.

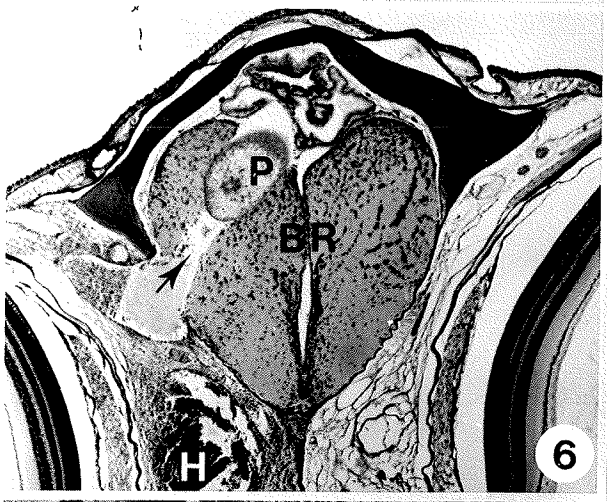
FIG. 10. Area of developing hooks (arrows) in scolex region of plerocercoid (P) at Day 75 PI. Note plerocercoid is in body cavity (B) rather than in muscle (M); H & E, X 160.



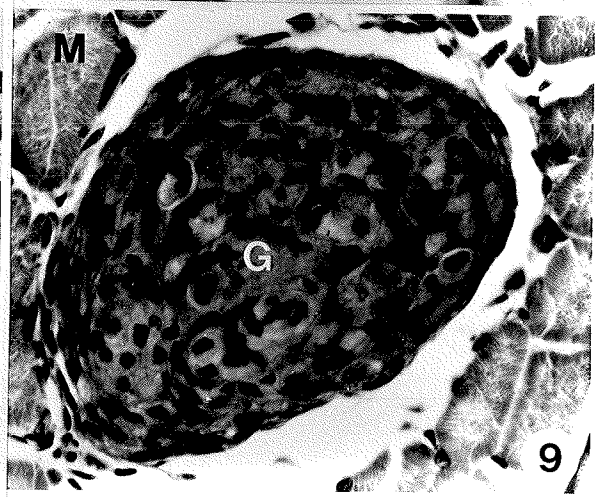
5



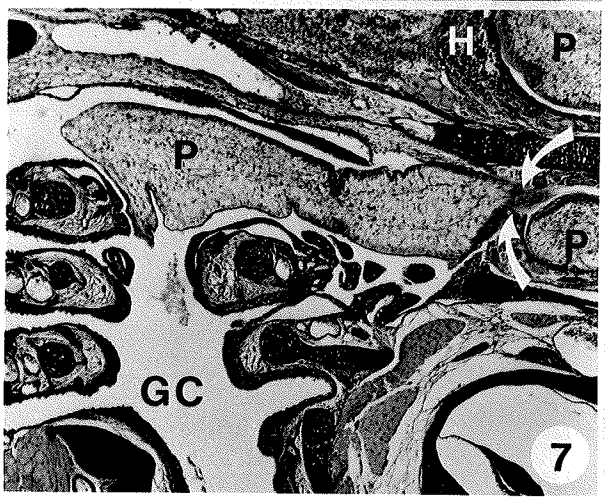
8



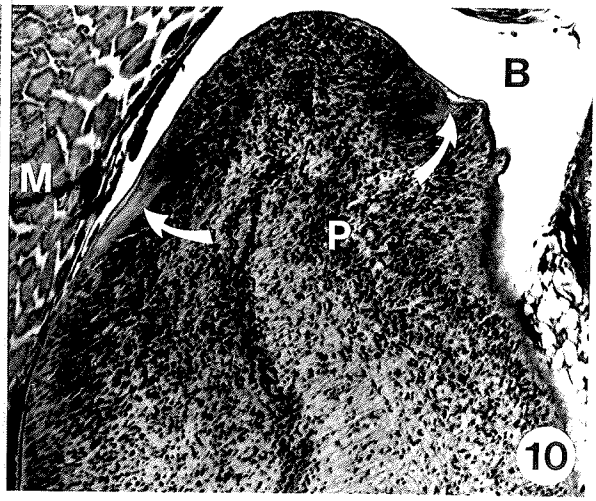
6



9



7



10

Swimming activity was reduced and a loss of equilibrium was evident for fish after Day 40 PI. Mortality of infected fish was first noted at Day 44 PI, and by Day 56 PI, 45% of infected trout died. The majority of dead trout had plerocercoids penetrating through their integument. There was no mortality of control fish during the experiment, and infected fish did not appear to be smaller than control fish.

Discussion

This study has clearly shown that plerocercoids of T. crassus become established and develop in rainbow trout. Complete differentiation of plerocercoids (i.e., fully-formed hooks) was not observed in trout, but this may be due to the age of infections (maximum 75 days) since fully-developed plerocercoids in whitefish fry were occasionally found as late as Day 100 PI at similar temperature (Chapter 2). The host response of trout to T. crassus during the early migration of the worm in muscle was similar to that in whitefish (Chapter 2), but hemorrhaging in live fish was later in trout ($\bar{X}$ = 37 Days PI) than in whitefish ($\bar{X}$ = 25 Days PI). Extra-muscular positions were more frequently occupied by plerocercoids in trout than in whitefish where epaxial and hypaxial muscles were the usual site (Miller 1952; Chapter 2). Plerocercoids in trout did not coil upon themselves and were not surrounded by a host capsule at Day 75 PI, although this was the usual situation in whitefish at this time (Chapter 2). Similarly, Kuperman (1973) found unencapsulated plerocercoids of T. crassus in salmon and T. orientalis in the Amur sleeper. These findings corroborate other experimental studies in which the host response to a particular species of helminth varied with the species of fish (Bylund 1972; Sommerville 1981). The high mortality of infected trout was similar to whitefish fry mortality (Dick and Rosen 1982; Chapter 2). Mortality occurred during the same period of time in trout and whitefish (i.e., 1.5 - 2 months PI), and coincided with the large increase in plerocercoid size at this time. The mortality observed during the muscle phase of T. crassus in experimentally infected trout is in contrast to Kuperman's (1973) opinion that plerocercoids of T. crassus are not harmful to the host.

The extra-muscular sites of plerocercoids, lack of capsule formation and high mortality of rainbow trout infected with T. crassus are probably indicative of an abnormal host that plays little or no role in natural transmission of this parasite. Nevertheless, the severe pathology and death attributed to even very low levels of infection in rainbow trout clearly established the importance of T. crassus as a pathogen to these fish. Furthermore, stocking of rainbow trout into aquatic systems known to have T. crassus should be carefully considered in light of this study.

GENERAL CONCLUSIONS

1. (a) Significant mortality of C. b. thomasi infected with the procercoid of T. crassus has been demonstrated.

   (b) The first peak of mortality occurred during the period of coracidium penetration into the haemocoel, while the second and larger peak coincided with the completion of most of the procercoid growth.

2. (a) A decrease in the mean intensity of infection over time indicated that cyclopid death was related to procercoid number.

   (b) The predominance of uninfected and lightly infected cyclopids after 28 days at 15°C was similar to the frequency of T. crassus reported from natural infections of cyclopids. Therefore, the low intensities of procercoids in natural systems may result from mortality of heavily infected hosts in addition to low coracidia densities.

3. (a) The intensity of infection was a factor which influenced procercoid size, differentiation and infectivity to the second intermediate host. There was a decrease in size and number of fully developed procercoids with increased parasite intensities suggesting intraspecific competition for space and or food. Light infections of cyclopids, in which procercoids were larger and a greater proportion fully differentiated, resulted in a a greater % recovery of plerocercoids from fish than when whitefish were exposed to heavily infected cyclopids.

   (b) Cyclopid sex and temperature were factors which influeced procercoid size. Procercoids were larger in adult female C. b. thomasi than in adult males, and this may be related to the

larger size of females and thus more available nutrients. The size of procercoids was smaller at 23°C than at 15°C in adult female C. b. thomasi.

4. (a) The majority of procercoids were in the cephalothorax of C. b. thomasi. Procercoids in the abdomen were rare, and smaller and less developed than those in the cephalothorax.

   (b) Procercoids tended to be directed anteriad in the cephalothorax and posteriad in the abdomen.

5. (a) The plerocercoid of T. crassus underwent a period of rapid growth during the first 60 days of infection, and most of this growth occurred in the host muscle.

   (b) Development of plerocercoid hooks occurred between Days 80-100 PI.

   (c) Plerocercoid movement in vitro up to Day 30 PI was the result of a series of peristaltic waves along the length of the worm's body. These movements probably occur in the muscle of fish, although at a reduced rate, and assist in its tissue migration.

   (d) Plerocercoids entered the body cavity of whitefish fry by 20 hrs. PI and the muscle by Day 5 PI where they underwent a two month period of growth, differentiation and migration. Plerocercoids were wound throughout the muscle by Day 60 PI and coiled upon themselves after this time.

   (e) Plerocercoids in the body cavity of whitefish fry after Day 5 PI were rare, and as shown by the experimental transplants and reconstruction of plerocercoid migrations, must have reentered this site from the muscle prior to the completion of their development and encapsulation by the host.

6. (a) Plerocercoids of T. crassus elicited a chronic inflammatory response from whitefish fry. Random migration of worms through the muscle resulted in hemorrhaging and an infiltration of inflammatory cells into vacated lesions which were transformed into granulomas by Day 60 PI.

   (b) The formation of a host capsule around plerocercoids was complete by Day 70 PI at 15°C. Capsule shape appeared to be influenced by the way plerocercoids coiled.

7. Some plerocercoids were alive at 3 years PI, but degeneration of some worms occurred by 2 1/2 years PI.

8. (a) Significant mortality of infected whitefish fry occurred near the end of the plerocercoid's rapid growth phase (Days 48-59 PI) when the worm was wound extensively through the musculature.

   (b) The fork length and liver glycogen of whitefish fry were unaffected by infection with T. crassus.

9. Rainbow trout were successfully infected with T. crassus in the laboratory.

10. Mortality of infected trout was higher than in whitefish, and the worm was less restricted to the muscle and was not encapsulated by Day 75 PI. The marked pathology and mortality observed in rainbow trout make it unadvisable to introduce these fish into T. crassus infested waters.

REFERENCES

Arnason, I.G. 1948. Infestation of Cyclops bicuspidatus with Triaenophorus at Heming Lake, Manitoba, during the summer of 1948. Fish Res. Board Can., Ann. Rept. of the Central Fisheries Research Station for 1948. App. 16:26-27.

Bliss, C.I. and R.A. Fisher. 1953 Fitting the negative binomial distribution to biological data and a note on the efficient fitting of the negative binomial. Biometrics. 9:176-200.

Bylund, G. 1972. Pathogenic effects of a diphyllobothriid plerocercoid on its host fishes. Commentationes Biologicae, Societas Scientiarum Fennica, Helsinki-Helsingfors. 58:1-11.

_____. 1975. Studies on the taxonomic status and biology of Diphyllobothrium vogeli Kuhlow, 1953. Commentationes Biologicae, Societas Scientiarum Fennica, Helsinki-Helsingfors. 79:1-22.

Clarke, A.S. 1954-55. Studies on the life cycle of the pseudophyllidean cestode Schistocephalus solidus. Proc. Zool. Soc. London. 124: 257-302.

Dick, T.A. and R. Rosen. 1982. Experimental infections of whitefish, Coregonus clupeaformis (Mitchill), with Triaenophorus crassus Forel. J. Fish. Dis. 5:83-86.

Finn, J.P. and N.O. Nielson. 1971. The inflammatory response of rainbow trout. J. Fish Biol. 3:463-478.

Guttowa, A. and W. Michajlow. 1964. Experimental infection of Copepoda from Tbilisi (USSR) with the larvae of Triaenophorus nodulosus (Pall.) from Mazurian lakes (Poland). Acta. Parasitol. Pol. 12: 195-199.

Halvorsen, O. 1967. The occurrence of procercoids of Diphyllobothrium norvegicum Vik in the abdomen of copepod hosts. Norweg. J. Zool. 14:142-146.

Hjortland, A.L. 1928. On the structure and life history of an adult Triaenophorus robustus. J. Parasitol. 15:38-44.

Humason, G.L. 1979. Animal Tissue Techniques. W.H. Freeman and Co., San Francisco. 4th Ed.

Janicki, C. and F. Rosen. 1918. Le cycle evolutif du Dibothriocephalus latus L. Societe Neuchateloise des Sciences Naturelles. Bulletin. 42:19-53.

Keymer, A.E. 1980. The influence of Hymenolepis diminuta on the survival and fecundity of the intermediate host, Tribolium confusum. Parasitology. 81:405-421.

Keymer, A.E. and R.M. Anderson. 1979. The dynamics of infection of Tribolium confusum by Hymenolepis diminuta: the influence of infective - stage density and spatial distribution. Parasitology. 79:195-207.

Kuhlow, F. 1953. Uber die Entwicklung und Anatomie von Diphyllobothrium dendriticum Nitzsch 1824. Z. Parasitenk. 16:1-35.

Kuperman, B.I. 1966. Eksperimental'noe issledovanie tsikla razvitiya Triaenophorus nodulosus (Pall., 1760) (Cestoda, Pseudophyllidea). Dokl. Akad. Nauk, SSSR. 169:1234-1235.

_____. 1973. Tapeworms of the Genus Triaenophorus, Parasites of Fishes. Nauka Publishers, Leningrad Sec., Leningrad. (English translation by Amerind Publishing, Co. Pvt. Ltd., New Delhi, 1981 for U.S. Dept. Int. and NSF, Washington, D.C.).

Kuperman, B.I. and V.G. Davydov. 1981. The fine structure of glands in oncospheres, procercoids and plerocercoids of Pseudophyllidea (Cestoidea). Int. J. Parasitol. 12:135-144.

Lawler, G.H. 1952. Host studies on Triaenophorus and the effect of Triaenophorus on growth. Fish. Res. Board Can., Ann. Rept. of the Central Fisheries Research Station for 1952. App. 1:23-25.

Lawler, G.H. and N.H.F. Watson. 1963. Measurements of immature stages of Triaenophorus. J. Fish. Res. Board Can. 20:1089-1093.

Lee, F.O. and T.C. Cheng. 1970. The histochemistry of Stellantchasmus falcatus Onji and Nishio, 1915 (Trematoda: Heterophyidae) metacercarial cyst in the mullet Mugil cephalus L. and histopathological alterations in the host. J. Fish Biol. 2:235-243.

Margolis, L., G.W. Esch, J.C. Holmes, A.M. Kuris and G.A. Schad. 1982. The use of ecological terms in parasitology (Report of an ad hoc committee of The American Society of Parasitologists). J. Parasitol. 68:131-133.

Margolis, L. and J.R. Arthur. 1979. Synopsis of the Parasites of Fishes of Canada. Bull. 199 Fish. Res. Bd. Can., 269 pp.

Meyer, M.C. and R. Vik. 1963. The life cycle of Diphyllobothrium sebago (Ward, 1910). J. Parasitol. 49:962-968.

Michajlow, W. 1953. O stosunkach wewnatrzgatunkowych w populacjach procerkoidow Triaenophorus lucii (Mull). Acta. Parasitol. Pol. 1:1-28.

Michajlow, W. 1962. Species of the genus Triaenophorus (Cestoda) and their hosts in various geographical regions. Acta. Parasitol. Pol. 10:1-38.

Miller, R.B. 1943. Studies on cestodes of the genus Triaenophorus from fish of Lesser Slave Lake, Alberta. II. The eggs, coracidia, and life in the first intermediate host of Triaenophorus crassus Forel and T. nodulosus (Pallas). Can. J. Res. 21:284-291.

_____. 1945a. Effect of Triaenophorus on growth of two fishes. J. Fish. Res. Board Can. 6:334-337.

_____. 1945b. Studies on cestodes of the genus Triaenophorus from fish of Lesser Slave Lake, Alberta IV. The life of Triaenophorus crassus Forel in the second intermediate host. Can. J. Res. 23: 105-115.

_____. 1952. A review of the Triaenophorus problem in Canadian lakes. Bull. Fish. Res. Board Can. No. 95.

Mount, P.M. 1970. Histogenesis of the rostellar hooks of Taenia crassiceps (Zeder, 1800) (Cestoda). J. Parasitol. 56:947-961.

Mueller, J.F. 1959a. The laboratory propagation of Spirometra mansonoides (Mueller, 1935) as an experimental tool. II. Culture and infection of the copepod host and harvesting the procercoid. Trans. Am. Microsc. Soc. 78:245-255.

_____. 1959b. The laboratory propagation of Spirometra mansonoides (Mueller, 1935) as an experimental tool. III. In vitro cultivation of the plerocercoid larva in a cell-free medium. J. Parasitol. 45:561-573.

Orr, T.S.C. and C.A. Hopkins. 1969. Maintenance of Schistocephalus solidus in the laboratory with observations on rate of growth of, and proglottid formation in, the plerocercoid. J. Fish. Res. Board Can. 26:741-752.

Petersson, A. 1971. The effect of lake regulation on populations of cestodan parasites of Swedish whitefish Coregonus. Oikos. 22:74-83.

Poole, R.W. 1974. An Introduction to Quantitative Ecology. McGraw-Hill Book Co., New York.

Price, J.L. 1958. Speciation in Cyclopidae and experimental infection of cyclopids with Triaenophorus nodulosus and T. crassus. Fish. Res. Board Can., Biol. Sta., London, Ontario (ex. Winnipeg, Man.), Manuscript Report Series (Biological) No. 640.

Puchtler, H., F.S. Waldrop and L.S. Valentine. 1973. Polarization microscopic studies of connective tissue stained with picro-sirius red F3BA. Beitr. Path. Bd. 150:174-187.

Roberts, R.J., A. MacQueen, W.M. Shearer and H. Young. 1973. The histopathology of salmon tagging I. The tagging lesion in newly tagged parr. J. Fish Biol. 5:497-503.

Rogers, D. E., L. Gilbertson and D. Eggers. 1972. Predator-prey relationship between arctic char and sockeye salmon smolts at the Agulowak River, Lake Aleknagik, in 1971. Univ. Washington, Fisheries Research Institute. Circular 72-7:1-40.

Rosen, F. 1918. Recherches sur le développement des cestodes. I. Le cycle evolutif des Bothriocephales. Societé Neuchateloise des Sciences Naturelles. Bulletin 43:241-300.

Sommerville, C. 1981. A comparative study of the tissue response to invasion and encystment by Stephanochasmus baccatus (Nicholl, 1907) (Digenea: Acanthocolpidae) in four species of flatfish. J. Fish Dis. 4:53-68.

Tanner, C. E., M. A. Curtis, T. D. Sole and K. Gyapy. 1980. The non-random, negative binomial distribution of experimental Trichinellosis in rabbits. J. Parasitol. 66:802-805.

Vik, R. 1957. Studies of the helminth fauna of Norway. I. Taxonomy and ecology of Diphyllobothrium norvegicum n. sp. and the plerocercoid of Diphyllobothrium latum (L.). Nytt Mag. Zool. 5:25-93.

Vogt, K. 1938. X. Experimentelle Untersuchungen über die Grunde von Masseninfektionen mit Plerocercoiden des Fischbandwurms Triaenophorus nodulosus (Pall.). Zeitschrift fuer Fischerei und Deren Hilfswissenschaften. 36:193-224.

Watson. N.H.F. and G. H. Lawler. 1965. Natural infections of cyclopid copepods with procercoids of Triaenophorus spp. J. Fish. Res. Board Can. 22:1335-1343.

Watson. N.H.F. and J.L. Price. 1960. Experimental infection of cyclopid copepods with Triaenophorus crassus Forel and T. nodulosus (Pallas). Can. J. Zool. 38:345-356.

Watson. R.A. 1977. Metazoan parasites from whitefish, cisco and pike from Southern Indian Lake, Manitoba: A preimpoundment and diversion analysis. M.Sc. Thesis, U. Manitoba.

Wedemeyer, G.A. and D.J. McLeay. 1981. Methods for determining the tolerance of fishes to environmental stressors. In, Stress and Fish, ed. A.D. Pickering. Academic Press, New York. 247-275.

APPENDIX I

Historical taxonomy of *Triaenophorus crassus*.

Phylum	Platyhelminthes
Class	Cestoda
Subclass	Eucestoda
Order	Pseudophyllidea
Family	Triaenophoridae Lonnberg, 1889
Genus	*Triaenophorus* Rudolphi, 1793
Syn.	*Tricuspidaria* Rudolphi, 1793
Species	*T. crassus* Forel, 1868
Syn.	*T. robustus* Olsson, 1893
Syn.	*T. tricuspidatus* morpha *megadentatus* Wardle, 1932
Other species	*T. nodulosus* (Pallas, 1781)
	T. stizostedionis Miller, 1945
	T. amurensis Kuperman, 1968
	T. meridionalis Kuperman, 1968
	T. orientalis Kuperman, 1968

APPENDIX II

Hatching times of *T. crassus* eggs held at 8°C and 15°C.

Coracidia Hatch+	Rosen (1983) 15°C			Kuperman(1973) 13-17°C	
	N*	$\bar{X}$±SD(Days)	Range(Days)	N*	Range(Days)
First Hatch	28	7.6±3.3	2-14	16	5-10
Maximum Hatch	18	12.8±5.6	4-22	16	6-11
Last Hatch	11	19.8±9.1	10-34	16	14
Duration Hatch	11	15.8±8.5	8-29	16	8

+ Hatch calculated from first day of egg laying
* N = number of cultures (Rosen), number of experiments (Kuperman)

Coracidia Hatch+	Rosen (1983) 8°C			Kuperman(1973) 8-12°C	
	N*	$\bar{X}$±SD(Days)	Range(Days)	N*	Range(Days)
First Hatch	26	10.0±5.5	4-26	10	7-10
Maximum Hatch	22	20.2±11.0	8-40	10	12-13
Last Hatch	14	41.9±24.6	8-81	-	-
Duration Hatch	14	33.7±21.2	4-70	-	-

+ Hatch calculated from first day of egg laying
*N = number of cultures (Rosen), number of experiments (Kuperman).

APPENDIX III

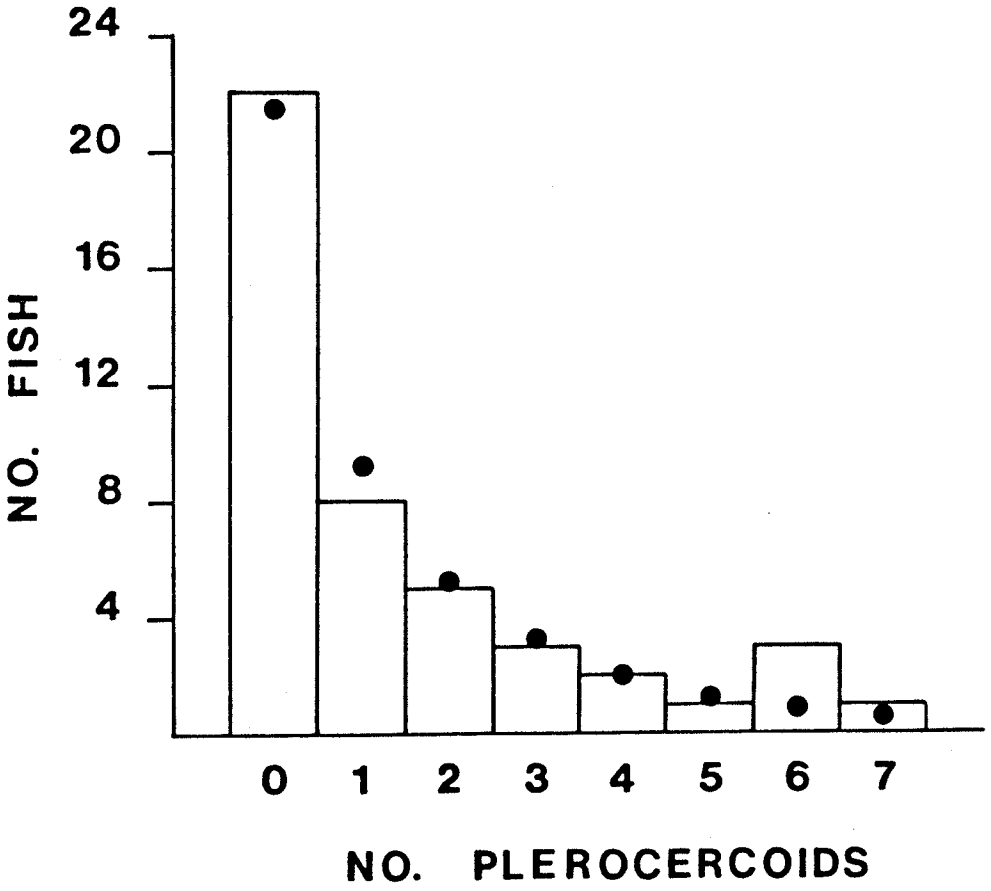
Condition of exposure of cyclopids and fish to T. crassus and recovery of procercoids and plerocercoids in 1980 pilot experiments.

Host (Age)	Cyclopid Infections				Fish Infections			
	Age(Days) Coracidia Culture	$\bar{X}$ Coracidia/ Cyclopid (Vol. H_2O)	Prev. Infect. (Day PI,N)*	x Intens. Infec. (Range)	Age(Days) Procer. (Temp.)	No. Procer. /Fish†	Prev. Infect. (N)§	$\bar{X}$ Intens. Infec. (Range)
Whitefish (2 months)	39-40(8°C)	48 and 82(7.5ml)	72.2(15-16,18)	2.1±1.0(1-4)	26(15°C)	1.5	26.7(15)	1.5(1-3)
Whitefish (2 months)	40(8°C)	80(7.5ml)	50.0(15,8)	2.0±1.4(1-4)	37(15°C)	2.0	13.3(15)	1(1)
Whitefsih (2 months)	39(8°C)	69(7.5ml)	33.3(16,9)	3.0±1.0(2-4)	40(15°C)	5.0	33.3(6)	1(1)
Whitefish (1 year)	8(8°C)	93(10.0ml)	100.0(16,4)	6.0±3.6(3-10)	21-24(15-26°C)	6.0	neg.(10)	———
	8(8°C)	18(10.0ml)	40.0(20,5)	3.5±3.5(1-6)				
	12(8°C)	46(10.0ml)	80.0(?,5)	2.8±1.7(1-5)				
Whitefish (2 years)	16(8°C)	25,000(800ml)	55.6(17,9)	1.4±0.6(1-2)	17(8°C)	?	neg.(15)	———
Whitefish (2 years)	39-40(8°C)	48 & 82(7.5ml)	72.2(15-16,18)	2.1±1.0(1-4)	21(15°C)	3.8T	neg.(20)	———
Whitefish (2 years)	4(8°C)	41(7.5ml)	66.7(14,6)	2.8±1.0(2-4)	42(15-26°C)	2.0T	neg.(3)	———
Rainbow trout (1 year)	4(15°C)	82(7.5ml)	100.0(14,4)	3.2±0.7(3-4)	35-38(15-26°C)	? T	neg.(15)	———
	4(15°C)	41(7.5ml)	75.0(14,4)	3.3±1.2(2-4)				
	6(?)	87(20.0ml)	50.0(35,8)	3.0±1.2(2-4)				
Rainbow trout (1 year)	39-40(8°C)	80(7.5ml)	63.2(15-16,19)	2.0±1.1(1-5)	32(15°C)	2.5T	neg.(15)	———

* N=number of cyclopids examined at Day N post-infection.
† Numbers are probably overestimated in most cases since they are based on the prevalence and intensity in younger infections. Mortality of infected cyclopids would have reduced prevalence and intensity by the time of fish infections.
§ Number of fish exposed.
T Fish which were exposed to infected cyclopids by gastric intubation.

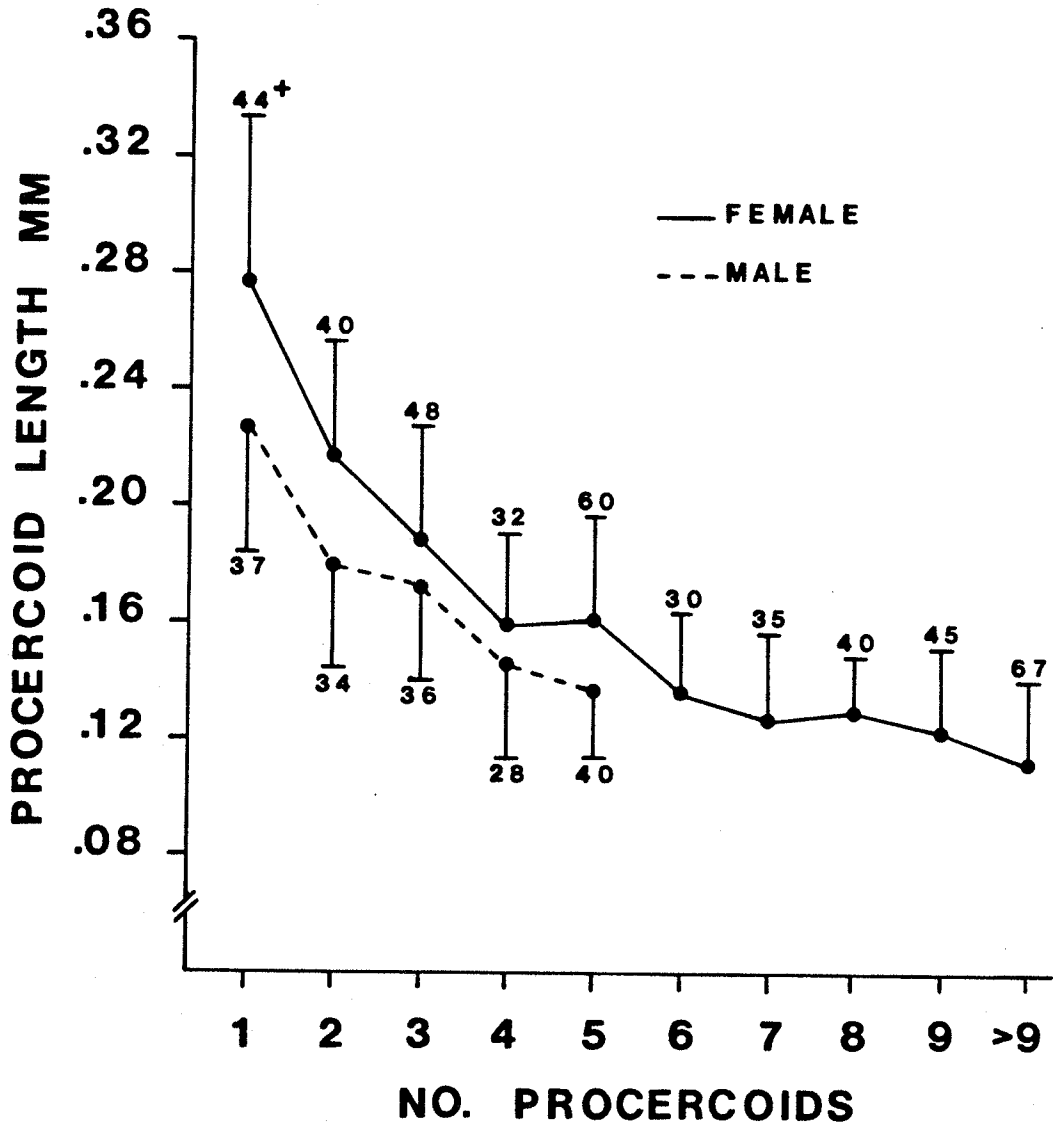
APPENDIX IV

Frequency distribution of *T. crassus* in experimentally infected whitefish fry (see Chapter 1, Table 1, Expt. 1). Fish were exposed as a group to an average of 3.4 procercoids each. (● = fitted negative binomial, $k = .615$, $x^2 = 1.228$, $P > .500$).


24
20
16
12
8
4
NO. FISH
0
1
2
3
4
5
6
7
NO. PLEROCERCOIDS

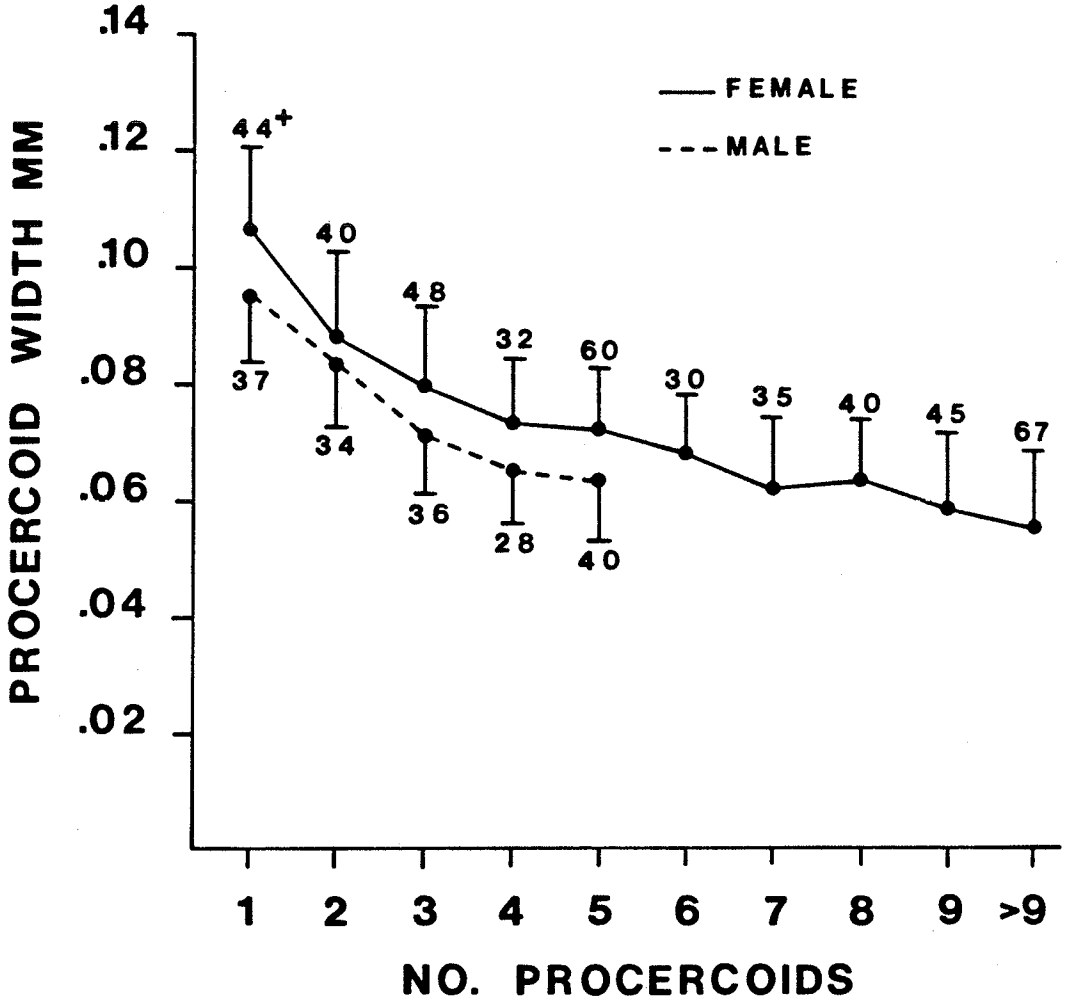
APPENDIX V

$\bar{X}$ length ± S.D. of *T. crassus* procercoids at 14-28 Days PI and at various levels of infection in adult male and female *C. b. thomasi*. Infections were maintained at 15°C. (+ no. procercoids measured).


.36
.32
.28
.24
.20
.16
.12
.08
PROCERCOID LENGTH MM
FEMALE
MALE
44+
40
48
32
60
30
35
40
45
67
37
34
36
28
40
1
2
3
4
5
6
7
8
9
>9
NO. PROCERCOIDS

APPENDIX VI

$\bar{X}$ width ± S.D. of *T. crassus* procercoids at 14-28 Days PI and at various levels of infection in adult male and female *C. b. thomasi*. Infections were maintained at 15°C. (+ no. procercoids measured).


.14
.12
.10
.08
.06
.04
.02
PROCERCOID WIDTH MM
FEMALE
MALE
44+
40
48
32
60
30
35
40
45
67
37
34
36
28
40
1
2
3
4
5
6
7
8
9
>9
NO. PROCERCOIDS

APPENDIX VII

Mortality in cyclopid groups exposed to coracidia of T. crassus.

DAY PI	PREVALENCE (N) ‡	$\bar{X}$ INTENSITY (RANGE)	% MORTALITY (N)+
13	90.0 (10)	3.4 ± 2.5 (1-7)	17.7 (300)
13	100.0 (10)	4.5 ± 2.3 (2-9)	51.0 (192)
14	37.5 (61)	2.2 ± 1.8 (1-7)	14.7 (75)
14*	30.0 (60)	1.4 ± 0.6 (1-3)	20.0 (75)
15	80.0 (10)	3.8 ± 1.6 (1-6)	42.2 (192)
16	72.7 (11)	5.4 ± 2.6 (2-8)	36.3 (344)
16	80.0 (10)	4.4 ± 4.5 (1-12)	43.6 (312)
18-19*	44.4 (9)	2.8 ± 1.0 (2-4)	74.1 (340)
20	58.3 (12)	5.4 ± 3.1 (3-12)	25.0 (288)
21	75.0 (8)	5.5 ± 1.8 (4-8)	25.3 (75)
21	23.9 (46)	1.8 ± 1.3 (1-5)	38.7 (75)
21*	30.2 (63)	1.8 ± 0.8 (1-3)	16.0 (75)
23-26	55.6 (9)	2.2 ± 1.6 (1-4)	77.4 (805)
28	24.1 (112)	1.5 ± 1.1 (1-5)	62.3 (300)
28	35.4 (79)	1.9 ± 1.1 (1-5)	72.3 (300)
28	18.9 (37)	2.7 ± 2.4 (1-7)	50.7 (75)
28*	26.5 (49)	1.5 ± 0.8 (1-3)	34.7 (75)
37	15.0 (20)	1.3 ± 0.6 (1-2)	64.3 (56)

* Cyclopids held at 23°C instead of 15°C.

‡ Number of cyclopids examined for determining prevalence and $\bar{X}$ intensity.

+ Number of cyclopids exposed to coracidia of T. crassus.

APPENDIX VIII

Criteria for classification of cellular components.

It is clear from Ellis's (1977) review article that there is much confusion regarding the nomenclature of fish leucocytes. Cell morphology and histochemical staining have been used to classify fish blood cells, but there is considerable variation in these parameters between fish species and the activity of most cells is poorly understood. This has often led to the naming of fish leucocytes after their mammalian equivalents without an understanding of their function.

The following criteria were used in the classification of whitefish blood cells, but under the limitations previously discussed. [Cells were observed in sectioned material from whitefish experimentally infected with T. crassus, and from smears of peripheral blood and kidney taken from uninfected fish].

A. Polymorphonuclear leucocyte (= PMN, neutrophil, heterophil and type 1 leucocyte) - These cells were up to 11 uM in diameter, had a lobed nucleus and were commonly associated with sites of inflammation in T. crassus infections. In smears of peripheral blood,cells had a reddish cytoplasm and a light purple nucleus when stained with PAS-hematoxylin, and a gray cytoplasm and a purple nucleus when stained with Giemsa. Granuloblasts, often without lobed nuclei, were found in kidney smears, and were probably precursors of PMN leucocytes in the peripheral circulation.

B. Macrophage - These cells were up to 13 uM in diameter, and possessed a vacuolated cytoplasm and a peripheral nucleus which was crescent-shaped. Macrophages were commonly associated with necrotic muscle in

experimental infections of whitefish with T. crassus, and similar cells were observed in kidney smears but not in peripheral blood smears.

C. Erythrocyte - These cells were 17 uM in length by 13 uM in width, and had a large central nucleus. Immature cells (i.e., haemoblasts) were observed in kidney smears and rarely in peripheral blood smears.

D. Lymphocyte - These were small cells up to 7 uM with a large round nucleus and a small rim of cytoplasm which stained bluish-gray with Giemsa. These cells were never observed to be associated with T. crassus infections within the whitefish muscle, and were only observed in peripheral blood smears.

Ellis, A. E. 1977. Leucocytes of fish: A review. J. Fish Biol. 11:453-491.