THE UNIVERSITY OF MANITOBA

SOURCES OF VARIABILITY IN LIFE-HISTORY CHARACTERISTICS OF THE ANNUAL PHASE OF *TRIAENOPHORUS CRASSUS* (CESTODA: PSEUDOPHYLLIDEA)

BY

ALLEN WILLIAM SHOSTAK

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TABLE OF CONTENTS

ABSTRACT

The life cycle of Triaenophorus crassus Forel, 1868 (Cestoda) has an annual phase (adult in pike Esox lucius L., egg and coracidium in fresh water, and procercoid in copepods Cyclops bicuspidatus thomasi Forbes) and a resting phase (plerocercoids in coregonid fishes). Little is known about transmission through the annual phase apart from hosts involved. This study evaluated host-parasite relationships and the resource base of T. crassus, interactions among helminths, and phenotypic variability in life-history characteristics pertaining to transmission of T. crassus in the annual phase. In natural infections of pike intestinal lumen space and attachment sites, but not nutrients, were limiting resources. Intestinal distribution of helminths showed intraspecific and interspecific interactions, affected in part by pathology caused by T. crassus. There was intraspecific competition by T. crassus for attachment sites. Controlled feeding of experimentally-infected copepods found nutrients limiting for procercoids. A low proportion of T. crassus produced eggs, but overdispersion of parasites concentrated parasite fecundity into relatively few pike. Offspring of T. crassus were maintained as separate lineages under controlled conditions to evaluate phenotypic variability in the following life-history characteristics: adult mass and lifetime fecundity, egg size and hatching characteristics, infectivity of coracidia, and procercoid growth and differentiation. Variability in size increased between the egg stage and the immature worm in pike, but decreased during adult maturation. Genetic sources of variability among lineages were not quantified but several environmental sources were evaluated: year of collection, host fish,

and intestinal attachment site of the adult of a lineage. Each environmental source affected a different group of characteristics. Life-history characteristics were compartmentalized into several groups, suggesting there was evolution of coadapted groups of characteristics in T. crassus as a response to independent selection pressures in various portions of its complex life cycle. Phenotypic variability in T. crassus is viewed as a transmission adaptation of a parasite with a basically synchronized life cycle to short-term environmental unpredictability.

ACKNOWLEDGEMENTS

This study benefited directly and indirectly from the contributions of many persons. I take pleasure in thanking them here.

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LIST OF TABLES

LIST OF FIGURES

Figure | Page

LIST OF APPENDICES

GENERAL INTRODUCTION

The cestode Triaenophorus crassus Forel, 1868 has a holarctic distribution (Lawler and Scott 1954; Kuperman 1973) and has been responsible for economic losses to the commercial fishing industry in Canada since 1931 when restrictions were placed on exports of infected fish to the United States (Newton 1932). Attempts to control levels of T. crassus have proved unsuccessful or impractical (Miller 1948, 1950; Miller and Watkins 1946; Lawler 1960, 1961, 1966) but have served to illustrate that populations of T. crassus are resistant to strong perturbations of the numbers of adult and larval worms in a system, and that apart from knowing the host species involved we still know relatively little about the underlying mechanisms involved in transmission of T. crassus.

This parasite has a complex life cycle (Miller 1952; Michajlow 1962; Kuperman 1973) that involves an adult worm in northern pike Esox lucius L., synchronized release of eggs into fresh water during the spring spawning run of the pike, hatch of a free-swimming coracidium from the egg, ingestion and growth to a procercoid larva in the hemocoele of cyclopid copepods that are the first intermediate host, and ingestion and growth to a plerocercoid larva in the musculature of salmoniform fishes that are the second intermediate host. Ingestion of plerocercoids by pike and subsequent growth into adult worms completes the life cycle. In Canada the first intermediate host is usually Cyclops bicuspidatus thomasi Forbes, and coregonid fishes serve as second intermediate hosts (Miller 1952; Lawler and Scott 1954; Watson and Lawler 1965).

There are two important aspects of the biology of T. crassus, that

affect transmission but have received little or no quantitative evaluation. The first aspect is the interaction of a parasite with its environment. The environment of a parasite includes the abiotic environment, involving factors such as temperature, light, and chemical surroundings, and the biotic environment, involving different host species infected during the life cycle as well as other helminths of the same or different species. The effects of the biotic environment of parasites on transmission in natural populations have received little direct study. Usually, field observations are interpreted relative to the findings of classic laboratory studies such as those of Read (1951), Holmes (1961, 1962), and Roberts (1961) without consideration of the multitude of confounding factors affecting natural populations. The second aspect is variability among individual parasites in life-history characteristics such as growth, fecundity, and infectivity. Individual variability is a fundamental concept in biology since it adapts populations to changing abiotic and biotic environmental conditions, yet relatively little is known regarding individual variability in parasite life-history characteristics: how extensive variability is, what causes it, how it is adaptive, or how it affects transmission? This is not a problem restricted to T. crassus as individual variability in most helminth species has received little attention.

The objective of this thesis is to (1) evaluate interactions of T. crassus with its abiotic and biotic environment, (2) evaluate the extent and possible sources of individual variability of T. crassus with respect to its life-history characteristics, and (3) consider the role of these two aspects on transmission of T. crassus. The

evaluation will be based on observations from natural and experimental infections.

For logistic reasons this study was limited to the part of the life cycle that I term the "annual phase", since it is generally acknowledged to be completed within about one year (Miller 1952; Michajlow 1962; Kuperman 1973): life of the parasite in the definitive host, the water, and the first intermediate host. This contrasts with the "resting phase" of the life cycle, in which plerocercoids that establish in the second intermediate host after ingestion of infected copepods may live for several years (Miller 1952; Rosen and Dick 1984). Several aspects of the "resting phase" were recently studied by Rosen (1983).

The organization of this thesis is as follows. First, the life of T. crassus within the definitive host is examined, since that is the start of the annual phase of the life cycle. The interactions of T. crassus with northern pike (chapter 1) and with other helminth species (chapter 2) are evaluated. Then, characteristics of T. crassus just prior to the critical period of egg release are evaluated (chapter 3). Second, I consider characteristics of the free-living stages (chapter 4). Third, the life of T. crassus within the first intermediate host is examined. This involves assessment of resource availability for the parasite within the copepod (chapter 5) and then several aspects of the infection process and subsequent growth and differentiation of procercoids (chapter 6). Finally, I evaluate adult T. crassus and their offspring regarding continuity of life-history characteristics throughout the entire annual phase of the life cycle (chapter 7).

CHAPTER 1: INTESTINAL PATHOLOGY

ABSTRACT

Pathology surrounding the attachment site of Triaenophorus crassus Forel in the intestine of northern pike (Esox lucius L.) is described. The scolex caused ulceration of the mucosa and lamina propria. Cellular infiltration and collagen deposition in the tissue surrounding the scolex raised the scolex into the lumen in isolated nodules, or produced a general thickening of the lamina propria. The lesion was well vascularized, with haemorrhaging and sloughing of epithelium at the lumenal surface, but with limited necrosis at the surface or within the lesion. Thickening of the lamina propria and circular muscle layer underlying regions with dense attachment sites produced an external swelling of the intestine and caused a decrease in the lumen to ca. one-fifth normal diameter. The nematode Raphidascaris acus was present within the degenerating lamina propria that surrounded attachment sites of T. crassus, but the cestode Proteocephalus pinguis was only found attached in areas with intact villi.

INTRODUCTION

A number of studies have described intestinal pathology induced by adult cestodes in fish. In general the pathology ranges from little or no damage, when the scolex is situated at the base of the villi (Miller 1943a; Bucke 1971; Mackiewicz et al. 1972; Kuperman 1973; Pronina and Pronin 1982) to a chronic inflammation with marked fibrosis and local loss of epithelium when the scolex is situated in deeper tissues (Williams 1960; Mackiewicz et al. 1972; Kuperman 1973; Korting 1977; McKinnon and Featherston 1982).

Extensive lesions around scoleces of Triaenophorus crassus were noted in the northern pike (Esox lucius) examined in this study. Previous descriptions of the pathology associated with attachment of T. crassus include inflammation and ulceration with haemorrhage (Kuperman 1973) and the formation of a connective tissue "hillock" or "tubercle" around the scolex (Miller 1943a; Kuperman 1973; Korting 1977). However, surprisingly little is reported on the extent of damage, the structure of the lesion, and the cells involved. The objective of this study was to describe gross and histopathological characteristics of the intestine of northern pike infected with T. crassus.

MATERIALS AND METHODS

Northern pike were collected by angling or gill netting at Quigly L. (54°53'N, 101°7'W), Heming L. (54°53'N, 101°7'W), and Southern Indian L. (56°47'N, 98°54'W) in northern Manitoba, Canada, during May-July of 1981 and 1982. Pike were killed by a blow to the head, and the intestine (pylorus to anus) was removed within 3 min of host death. Some intestines were preserved by quick-freezing, in ethanol cooled to ca. -70C by addition of dry ice, for later necropsy and measurement of intestinal thickness. Other intestinal tracts had 5-mm thick transverse sections removed and fixed in 10% buffered formalin (Humason 1979) for histopathology studies and scanning electron microscopy (SEM). Gross pathology was described from fresh material.

Frozen intestines were processed as follows: (1) The intestines of one group (15 pike) were divided into 20 equal sections between the pylorus and intestinal-rectal valve, and 3 between the intestinal-rectal valve and the anus. Sections were numbered 1-23, with section 1 closest to the pylorus. The species and number of helminths attached in each section was recorded. A transverse cut was made through the midpoint of each section, and the maximum thickness of the intestinal wall (serosa to lumenal margin of the stratum compactum) measured using a dissecting microscope with ocular micrometer. (2) The intestines of a second group (11 pike) were cut into 23 sections

as above, and photographs taken of the cut surfaces. Total cross-sectional areas of the intestine, and of lumen only (the area central to the stratum compactum) were measured from the photographs.

The formalin-fixed material was prepared for SEM or light microscopy. Material for SEM was transferred to 70% ethanol, dehydrated to absolute ethanol, replaced with acetone, and critical-point dried using liquid CO_2 in a Sorval critical point drying unit. The dried material was trimmed with a razor blade to expose scoleces in situ, and then gold coated in a Balzer sputtering apparatus. Material was viewed in a Cambridge Stereoscan Mk IIA. Materials for light microscopy were embedded in paraffin and sectioned at 5-10 micrometers (μm). Sections were stained with haematoxylin and eosin (H and E), or azan stain, Mallory-Heidenhain (Humason 1979). Some sections were stained with the Lendrum acid picro-Mallory method for fibrin (Humason 1979), whereas others stained with picro-Sirius red F3BA (Puchtler et al. 1973) were viewed with polarized light to identify collagen.

Cells were identified on the basis of the terminology and descriptions of Roberts (1978) and Groman (1982). The tissue layers of the intestine were named using the terminology of Bucke (1971). The identity of T. crassus in tissue section was determined by the large scolex width (1.0 mm) and hook width (255-300 μm) which distinguish it from other North American triaenophorids (Kuperman 1973).

RESULTS

GROSS PATHOLOGY

The intact gut of infected pike was swollen and firm in the area of attachment by T. crassus but was usually not discolored. Transverse cuts through the center of the swelling revealed a thickening of the muscle and connective tissue layers (Fig. 1) and a decrease in lumen diameter. The appearance of the lumenal surface in fresh material differed depending on the density of attachment sites of T. crassus. Isolated attachment sites were characterized by a small nodule around the scolex, with villi absent from around the rim of the nodule but with surrounding villi intact. In areas of dense (> 10/cm^2) attachment sites (Fig. 2A) villi were absent around each scolex and between worms. The tissue exposed by loss of villi was cream-colored with a slightly pinkish tinge in fresh samples and the largest such area was ca. 66x10 mm. The scoleces of T. crassus were embedded in pits ca. 1 mm deep (Fig 2A) but in one instance a scolex had penetrated ca. 5 mm through mucosa, lamina propria, and muscle layers, almost reaching the serosa.

Figure 1. Thickness of the intestinal wall of northern pike at evenly-spaced intervals between the pylorus (section 1) and anus (section 23). Thickness was measured from serosa to lumenal margin of the stratum compactum. Thin lines, $\bar{X} \pm SD$ from eight pike not infected with T. crassus. Numbers indicate number of scoleces attached in a section (absence of a number means none attached). A. A single, narrow zone of attachment. B. A single, long zone of attachment. C. Two zones of attachment.

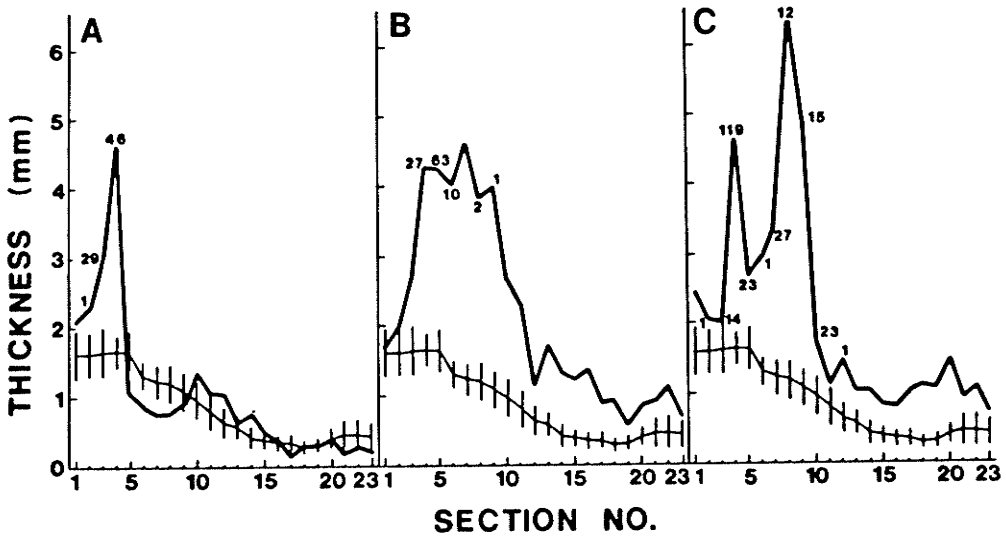
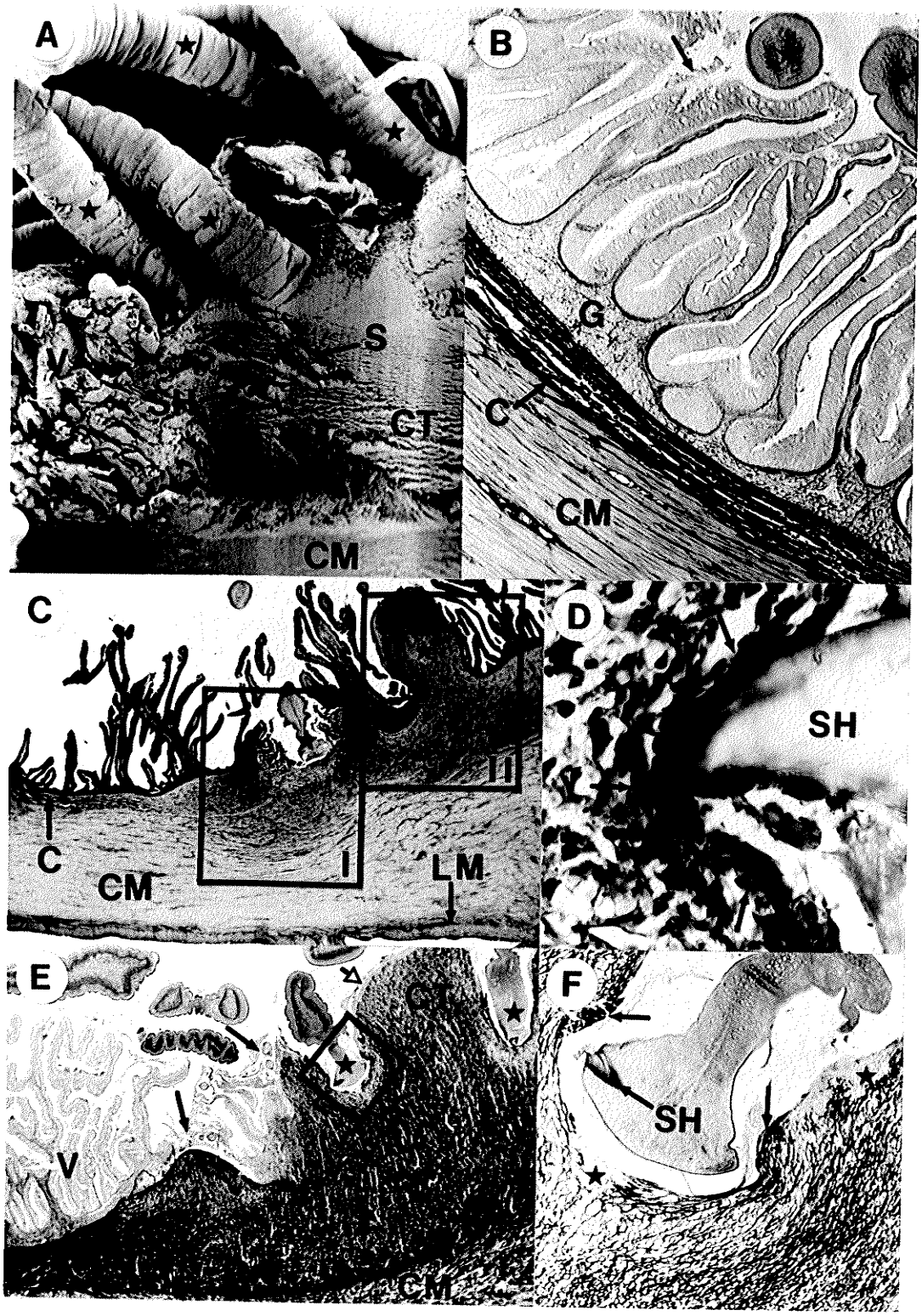

A
B
C
THICKNESS (mm)
SECTION NO.
0
1
2
3
4
5
6
1
5
10
15
20
23
46
29
1
27
63
10
2
1
119
12
15
27
1
23
14
23
1

Figure 2. Histopathology of northern pike intestine. A. Scanning electron micrograph of a longitudinal section of intestine in region of T. crassus (*) attachment. Note scolex (S) and scolex hook (SH) embedded within connective tissue pad (CT). Adjacent villi (V) are undamaged. CM, circular muscle layer (x72). B. Cross section of intestine posterior to region of T. crassus (*) attachment. Note damage to villi (arrow) close to strobila. G, stratum granulosum; C, stratum compactum; CM, circular muscle layer (picro-Sirius red, x75). C. Cross section of intestine showing undamaged tissue (left), with rectangle enclosing type I and type II lesions around scoleces of T. crassus (*). C, stratum compactum; CM, circular muscle layer; LM, longitudinal muscle layer (H&E, x13). D. Scolex hook (SH) of T. crassus within lesion in pike intestine, showing compression of cells (arrows) (H&E, x635). E. Cross section of intestine showing undamaged villi (V), and a type III lesion surrounding two T. crassus (*). Note absence of connective tissue from the lesion margin (open arrow), and damaged villi at the lesion periphery (closed arrows). CM, circular muscle layer; CT, connective tissue. Square is enlarged in Fig. 2F (picro-Sirius red, x13). F. Enlargement of T. crassus scolex in square from Fig. 2E. Note heavy deposits of collagen (arrows) around scolex hooks (SH), and areas where collagen is not deposited at edge of lesion (*) (picro-Sirius red, x60).


A
S
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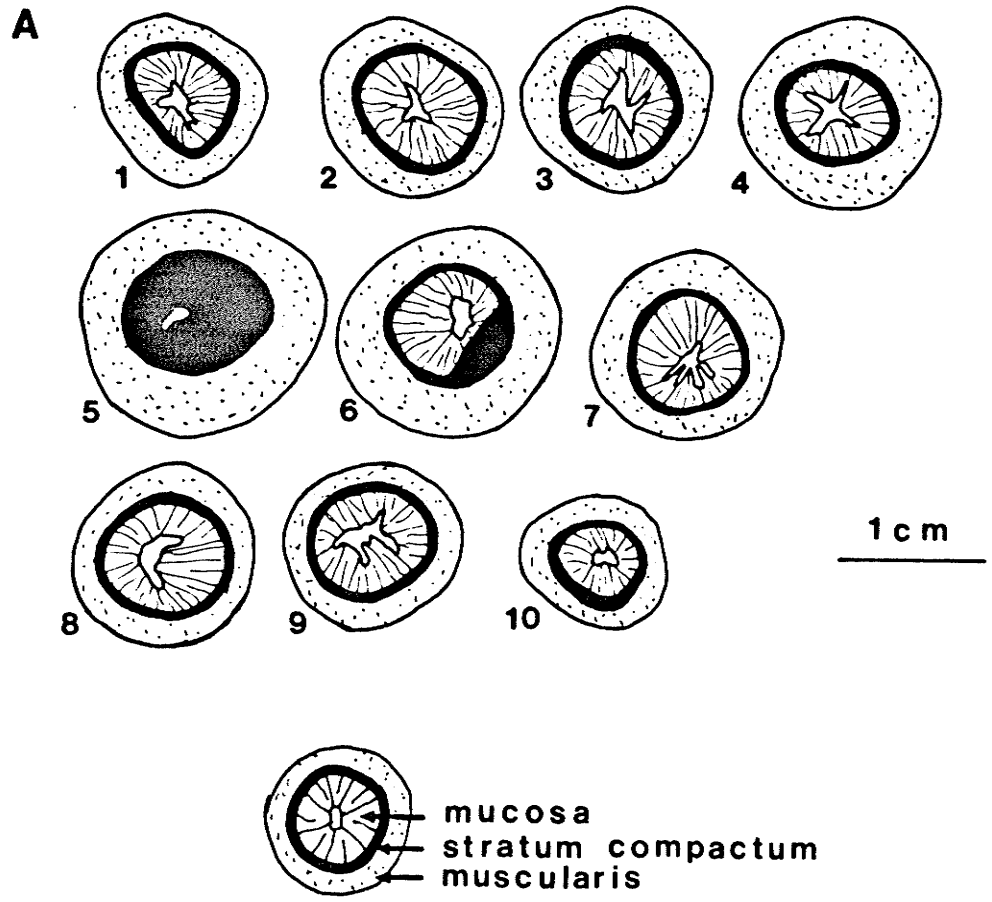
CT
CM
F
SH

Necropsy revealed the presence of 11 species of helminth (see Chapter 2). There was no pathology in the gut of pike when other helminths were present but T. crassus was absent. The highest density of T. crassus in a pike was 119 worms attached in a 17-mm-long section of intestine.

Intestines of pike not infected with T. crassus had uniformly thick walls in the anterior five sections, with a gradual thinning towards the rectum (Fig. 1A-C). The intestines of pike with more than a total of ca. 70 T. crassus were thicker at the attachment sites of the parasites (Fig. 1A-C) but this thickening of the intestine was not seen in lighter infections of T. crassus. Most infections were characterized by a single, narrow zone of attachment by T. crassus (Fig 1A). At higher intensities the attachment sites of T. crassus were often in a wider zone (Fig 1B) or were found in two separate aggregations (Fig. 1C). The thickness of the intestine generally increased as numbers of T. crassus attached in each section increased, but was also influenced by numbers attached in adjacent sections. Sections through attachment sites of T. crassus (Fig. 3) revealed that up to 95% of the normal cross-sectional area of the lumen was occluded. The degree of occlusion varied within and between fish, but two of six infected pike from Southern Indian L. had lumens 1.2 and 3.2 mm in diameter, compared with an average diameter of 8.1 mm (a 2.5- to 6-fold change) measured at corresponding sections in five uninfected pike of similar size. The connective tissue layer in the most severely occluded intestine was 5.1 mm thick, compared to a normal thickness of ca. 0.2 mm.

Figure 3. Drawings from photographs of transverse cuts through the 10 anterior sections of intestine in two northern pike. Each section was 5% of total intestinal length. A. A pike from Southern Indian Lake, Manitoba, with ca. 100 T. crassus attached in sections 4-7. B. A pike from Heming Lake, Manitoba, not infected with T. crassus.


A
1
2
3
4
5
6
7
8
9
10
1 c m
mucosa
stratum compactum
muscularis
B
1
2
3
4
5
6
7
8
9
10
1 c m

HISTOPATHOLOGY

Posterior to attachment sites the intestine had five layers (Fig. 2B,C). The mucosa consisted of columnar epithelial cells, numerous goblet cells, and occasional rodlet cells. The lamina propria had a thin, cellular stratum granulosum overlying about five thick connective tissue bands (the stratum compactum). The circular muscle layer was thick and the longitudinal layer was thin. The intestine was bounded externally by the serosa. Villi were intact except for slight damage to the tips where the epithelium was in close proximity to strobila of T. crassus (Fig. 2B).

The scolex penetrated through the epithelium and into the lamina propria, causing an ulceration at the point of attachment (Figs. 2C,E,F). Three categories of pathology were observed, based on the locations of the scolex in sectioned material. Type I lesions were shallow ulcers with the scolex situated within the stratum compactum and close to the circular muscle layer (Fig. 2C). Villi were present in the area surrounding the attachment site, but the stratum compactum was thickened due to infiltration of fibroblasts and leucocytes between the thick connective tissue bands. Cellular infiltration primarily involved fibroblasts, but some erythrocytes, leucocytes, and macrophages were found throughout the circular muscle layer. Type II lesions were ulcers in which the scolex was within a connective tissue nodule elevated into the lumen but surrounded by intact villi (Fig. 2C). The thick connective tissue bands of the stratum compactum were indistinct beneath or surrounding the attachment site (Fig. 2C), but

the connective tissue of the nodule was a fine meshwork of fibers. The circular muscle layer beneath the attachment site was thickened by collagen deposition, but there was less cellular infiltration than beneath type I lesions. Type III lesions were ulcers which contained several scoleces (Fig. 2A) in a thick meshwork of connective tissue, with complete loss of epithelium (Figs. 2E,F). The underlying muscle layer had more connective tissue than adjacent muscle beneath intact villi, but there was little cellular infiltration. The thickness of the circular muscle layer underlying type III lesions was variable: usually less than 20% thicker than adjacent muscle, but occasionally twice as thick.

Type I and II lesions were usually seen where there was a low density of attachment sites, or on the periphery of areas with dense attachment sites. Type III lesions were only seen in areas of dense attachment sites.

The structure of the mucosa and lamina propria varied with distance from the attachment site. These changes will be described in order from the most peripheral responses, to those in the immediate vicinity of the scolex.

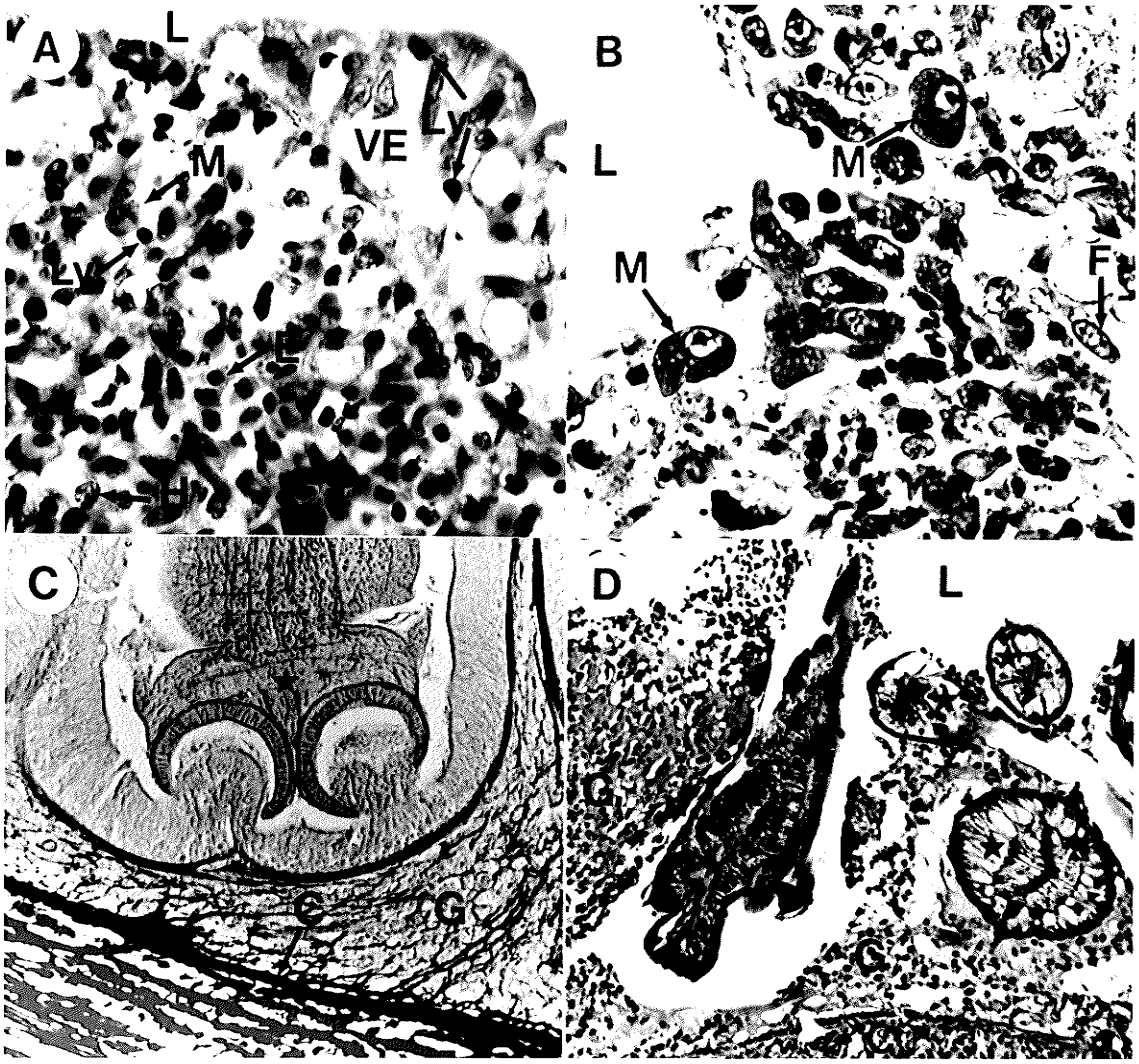
The most peripheral response was the cellular infiltration of the stratum compactum (described earlier) and hyperplasia of the stratum granulosum (Fig. 2C) which resulted in thickening of the lamina propria. Goblet cells were numerous in the mucosa adjacent to attachment sites. Macrophages, lymphocytes, and granulocytes were present within the loose connective tissue of the stratum granulosum. This connective tissue stained lightly with picro-Sirius red and

exhibited weak birefringence when viewed with polarized light.

Mucosa at the edge of lesions comprised vacuolated epithelial cells interspersed with lymphocytes, and lymphocytes as well as macrophages and heterophils were observed in the underlying tissue (Fig. 4A). Erythrocytes were free within the tissue, but the surface of the lamina propria exposed by loss of epithelial cells showed little evidence of sloughing or hemorrhaging (Fig. 4A).

Two layers were distinguished in the lamina propria surrounding attachment sites where the epithelium was lost (Fig. 2E,F) but it was not clear whether these layers were modified stratum compactum and stratum granulosum. From the circular muscle layer to within ca. 75 μm of the lumen the lesion was infiltrated with connective tissue (Fig. 2E,F) and was well-vascularized throughout. Macrophages and lymphocytes were present. Eosinophilic granular cells were less common, but contained larger granules than those in the lamina propria underlying intact mucosa. Pyknotic nuclei were dispersed throughout the lesion. Intact blood vessels and connective tissue were absent within about 75 μm of the lumen but the remaining tissue was intact and contained numerous lymphocytes, macrophages, and fibroblasts (Fig. 4B). Macrophages at the margins of a lesion (Fig. 4B) were larger than those occuring in deeper tissue. The tissue at the lumenal surface was sloughed into the lumen and contained large numbers of erythrocytes, lymphocytes, and pyknotic nuclei, but smaller numbers of macrophages. The matrix of the sloughed material was generally clear and did not stain for collagen with picro-Sirius red or Mallory's, but portions of it stained positively for fibrin.

Figure 4. Histopathology of northern pike intestine. A. Peripheral region of lesion, similar to area indicated by solid arrows in Fig. 2E, showing damage to epithelium. E, erythrocytes; H, heterophil; L, lumen; Ly, lymphocytes; M, macrophage; VE, vacuolated epithelial cell (H&E, x770). B. Large macrophages (M) near lumenal surface (L) of lesion. F, fibroblast (H&E, x770). C. Proteocephalus pinguis (*) attached at base of villus. G, stratum granulosum; C, stratum compactum (picro-Sirius red, x185). D. Raphidascaris acus (*) within stratum granulosum (G) at periphery of lesion, similar to the area indicated by the solid arrow in Fig. 2E. The stratum granulosum and stratum compactum (C) are distinct, but the overlying epithelial cells are lost, exposing the lamina propria to the lumen (L) (H&E, x185).


A
L
M
VE
Ly
Ly
B
L
M
M
F
C
C
G
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G

Scoleces were situated in pits near the lumenal surface of the lesion (Fig. 2E,F). Hooks compressed the surrounding cells (Fig. 2D) and some pyknotic and karyolytic nuclei were found in the tissue surrounding the scolex, but there were no other signs of necrosis. Polarized light was used to confirm that the stained material in Fig. 2E,F was collagen. Although collagen was not deposited at the edge of the lesion towards the lumen (Fig. 2E,F) it was present in thick deposits lining the pits, particularly around the hooks (Fig. 2F).

Other helminths were revealed in tissue section. The cestode Proteocephalus pinguis (Fig. 4C) was found attached at the base of intact villi only, but there was no evidence of tissue damage or cellular infiltration. Sections adjacent to attachment sites of T. crassus in which the epithelial layer was lost revealed the nematode Raphidascaris acus within the degenerating stratum granulosum, but not within the intact stratum compactum (Fig. 4D). Sections of R. acus were also observed in the lumen and at the surface of type II lesions (Fig. 2E). Larval R. acus were also observed at necropsy within the pits surrounding scoleces of T. crassus.

DISCUSSION

Pathology of the pike intestine caused by attachment of T. crassus differs somewhat from that described for other fish-parasite systems. These differences are due to the size of the scolex and hooks, the nature and extent of the lesion, and the biology of the parasite. However, before discussing these differences in detail, it is important to note the similarities the pike - T. crassus system has with other fish-parasite systems: (1) The severity of pathology appears largely due to the type of tissue damaged. Helminths which damage only the epithelium and stratum granulosum induce a minor response (Bullock 1963; Chaircharn and Bullock 1967; Bucke 1971; Mackiewicz et al. 1972; Kuperman 1973; Hine and Kennedy 1974). In contrast, helminths such as T. crassus which cause tissue breakdown as deep as the stratum compactum and/or the underlying muscle layers induce a chronic inflammatory reaction leading to extensive deposition of collagen around the scolex or proboscis (Chaircharn and Bullock 1967; Mackiewicz et al. 1972; Kuperman 1973; Hine and Kennedy 1974; McKinnon and Featherston 1982). (2) The cellular components of the repair process were similar to those reported in other studies (Bullock 1963; Roberts et al. 1973a,b; Hine and Kennedy 1974). The well-vascularized tissue around the scolex in type III lesions in this study was similar to the fibro-granulation tissue involved in repair of skeletal muscle lesions in the studies of Finn and Nielson (1971)

and Roberts et al. (1973a,b). Eosinophilic granular cells were present in the fibro-granulation tissue of pike, but were not as common as reported by others (Bullock 1963; Roberts et al. 1973a,b). The granules of these cells within the lesion in pike were larger than those from normal lamina propria, whereas Roberts et al. (1973b) reported that in salmonid fishes the granular cells in lamina propria and from repair tissue were indistinguishable. (3) The widespread loss of epithelium around the attachment sites in heavy infections was similar to the infections with Pomphorhynchus laevis described by Hine and Kennedy (1974).

The age of lesions around attachment sites of T. crassus in pike was unknown as infections were acquired naturally. However, it is likely that type I, II, and III lesions occur in that sequence, based on the increasing structural dissimilarity of tissue around the attachment site compared to normal tissue, the decrease in cellular infiltration coupled with increased collagen deposition in muscles underlying the attachment site, and increased distance between the scolex and the circular muscle layer.

There were four unusual features of pathology caused by T. crassus: (1) The deposition of collagen by fibro-granulation tissue in type II and III lesions elevated the scolex into the lumen, leaving a thick pad of connective tissue between scolex and intestinal muscles. This is atypical, as other reports indicate that connective tissue surrounds the scolex or proboscis but leaves the attachment organ near its original point of penetration (Chaicharn and Bullock 1967; Mackiewicz et al. 1972; Hine and Kennedy 1974; McKinnon and

Featherston 1982). (2) Accumulations of necrotic material were not observed in the pits surrounding scoleces. This differs from attachment sites of caryophyllid cestodes (Mackiewicz et al. 1972) but may be a function of pit depth only. (3) The epithelium was absent from extensive areas in type III lesions but the underlying tissue appeared healthy. Intact blood vessels occurred close to the intestinal lumen, and a majority of cells sloughed at the surface was intact. (4) There was a generalized host cellular response plus a localized response to the hooks. Deposits of collagen were thickest directly around the hooks and thinner in areas where cestode strobila contacted host tissues. The role of hooks as irritants was suggested by Korting (1977).

The level of pathology induced by caryophyllid cestodes in their hosts is correlated with the presence or absence of attachment organs on their scoleces (Mackiewicz et al. 1972). By contrast, a review of the literature on Triaenophorus spp. in northern pike (Miller 1943a; Kuperman 1973; Korting 1977; Pronina and Pronin 1982) and this study indicate that the level of pathology is correlated with the size of scolex and hooks. Triaenophorus nodulosus and T. amurensis have small scoleces and hooks and induce small, shallow ulcers, whereas T. crassus, T. meridionalis, and T. orientalis have large scoleces and hooks and cause extensive ulcers with marked fibrosis. For Triaenophorus spp. a single scolex design varying primarily in size can induce varied responses in a single host species. Consequently, the presence or absence of attachment organs, and their size, contribute to the level of pathology induced by cestodes in their fish hosts.

Triaenophorus crassus is not currently considered a pathogen of pike (Kuperman 1973), but the heavy infections observed in the present study caused a reduction in the diameter of the intestinal lumen which could lead to blockage by large undigested food items such as otoliths or vertebrae and restrict the normal flow of intestinal contents. Loss of epithelium may reduce absorptive surface and cause fluid loss. It would be interesting to examine the growth rates of pike from systems with and without T. crassus, particularly where its levels are high.

Although the host response was severe, some features of it may be considered adaptive to the pike. The deposition of connective tissue beneath the attachment site removes the scolex from proximity to deeper tissues, and thereby reduces inflammation and scarring of underlying muscle layers. In addition, gravid T. crassus detach from the intestinal wall and pass out, usually during pike spawning. There is a subsequent period of about one month with a minimal rate of reinfection (Miller 1943a; Kuperman 1973) when healing probably occurs. The maintenance of well-vascularized fibro-granulation tissue around the attachment site during the infection may permit rapid re-establishment of epithelium once the attachment site is vacated.

Not only was the pike - T. crassus system unusual regarding its pathology, but the changes induced by large numbers of T. crassus affected the pike helminth community. Parasite species which require intact villi for attachment, such as T. nodulosus (Miller 1943a) and Proteocephalus spp. (Andersen 1979; this study) were excluded from areas with type III lesions. Furthermore, R. acus, which was normally

found free in the lumen, was often lying within degenerating lamina propria near lesions caused by T. crassus. Pathology induced by large numbers of T. crassus was clearly a dominant factor in determining the availability of attachment sites for other parasites. This aspect will be examined in more detail in the next chapter.

CHAPTER 2: ECOLOGY OF THE INTESTINAL HELMINTH COMMUNITY

ABSTRACT

A natural experiment was conducted to study the structure of the intestinal helminth community of northern pike (Esox lucius). Evidence for structure was the presence of a deterministic group of helminth species, predictable sequences of colonizations and extinctions, predictable patterns of spatial distribution of helminths within the intestine, and the presence of interspecific interactions. The contributions of environmental heterogeneity, species-specific attributes, resource availability, and intraspecific and interspecific interactions to community structure were evaluated. Environmental heterogeneity influenced species composition, population sizes, and dispersion patterns of the helminths. Species-specific attributes such as choice of intermediate hosts, phenology, adaptations to different intestinal regions, and a tendency towards intraspecific aggregation made a large contribution to structure. Indirect estimates indicated that nutrient resources were not limiting, and direct estimates of attachment site and lumen space resources indicated these were only temporarily limiting for some species. Intraspecific competition for attachment sites limited numbers of the cestode Triaenophorus crassus. Other intraspecific interactions and all interspecific interactions involved displacement within the intestine, but competition was not inferred since there were no detectable alterations of the survival status of the participating

species. A combination of low population sizes of the component species in naturally-occurring infections, with the general abundance of three important resources, indicated that community structure of pike helminths was non-competitive in nature. From the current observation of distribution patterns of pike helminths, resource abundances, and types of interactions, it is unlikely that present-day interspecific competition makes a major contribution to community structure, and the conditions that would result in natural selection to minimize competition are probably rare.

INTRODUCTION

Several helminth species in addition to Triaenophorus crassus form a community within the intestine of northern pike. The previous chapter suggested the presence of interactions between T. crassus and other helminths, but was only able to provide limited characterization of those interactions. The parasitization of the pike intestine is the only point during the annual phase of the life cycle of T. crassus in which intraspecific or interspecific interactions are likely to occur and therefore warrants detailed study. The interactions occurring within a community define its structure, and it is therefore appropriate to evaluate infections of T. crassus in the northern pike in the context of community structure.

The nature of parasitic infections has neccessitated formation of a hierarchy for community classification. The individuals of all species occurring within a single host individual form an infracommunity, whereas all individuals of those species associated with the host population form a component community (Bush and Holmes 1986b). Interactions occur at the level of the infracommunity, and the use of the term community in the remainder of this chapter will specifically indicate the infracommunity level of organization.

Structure in natural communities may be provided through competition (Connell 1980; Schoener 1983; Wilson 1983; McAuliffe

1984), predation and disturbance (Dayton 1971; Paine 1984; Sousa 1984; Werner 1984), facilitation (Seifert and Seifert 1979), and species-specific preadaptations to these processes (Wiens 1977; Connell 1980; Price 1980,1984). The importance of these processes in natural communities varies due to diversity of geographic location, resource availability, and modes of resource exploitation by the component taxa (Price 1984). Seifert (1984) suggested that the identification of structuring factors for one community may at least be generalizable to phyletically-similar communities in similar environments.

Helminth communities found within the intestines of vertebrate hosts appear to be structured (Schad 1963; Butterworth 1982; Lotz and Font 1985; Bush and Holmes 1986b) but the mechanisms generating structure are unclear. Experimental studies employing controlled, two-species infections of mammalian hosts have implicated competition for nutrients (Read and Phifer 1959; Holmes 1961, 1962) or habitat modifications mediated through host defense responses (Howard et al. 1978; Silver et al. 1980; Ferretti et al. 1984) as mechanisms of interspecific interaction. The structure of the more diverse helminth communities found in nature apparently forms a continuum from interactive to primarily non-interactive (Holmes 1973; Rhode 1979; Price 1980, 1984; Butterworth 1982; Kennedy 1985; Lotz and Font 1985; Bush and Holmes 1986b). These classifications result from analyses of site occupation by intestinal helminths in "natural experiments", in which the response of one species is evaluated in a variety of environments (=hosts) where other species are either absent or present in varying numbers, due to the logistic problems involved in experimentally manipulating diverse natural helminth communities.

Many natural experiments on helminth communities have been done (Chappell 1969; Hair and Holmes 1975; Riley and Owen 1975; Grey and Hayunga 1980; Butterworth 1982; Kennedy 1985; Lotz and Font 1985; Bush and Holmes 1986b). Although aspects of helminth acquisition were generally considered, the characteristics of resource availability and utilization that may mediate interactions were extrapolated from laboratory results with little attempt to assess their validity in natural situations. The evolution of parasite communities, currently under debate (Holmes 1973; Rohde 1979; Price 1980, 1984), is but one topic that may be clarified by an integrated study of community structure and resource utilization under natural situations.

The structure of the helminth community of northern pike was studied using a mechanistic approach (Schoener 1986), that emphasizes the population and individual ecology of the component species. This seemed appropriate for studying a parasite community in which non-interactive mechanisms may be prominent. The objectives of this study were to: (1) evaluate sources of temporal and geographic heterogeneity in the composition of the pike helminth community, (2) assess the roles of attachment sites, nutrients, and space as potential limiting resources, (3) identify intra- and interspecific interactions, and (4) determine the contribution of each of these to community structure.

MATERIALS AND METHODS

STUDY AREA

Seventeen collections of northern pike were made in Manitoba, Canada, during 1981-1984, in order to maximize potential sources of geographic and environmental heterogeneity (Table 1). Five collection sites were chosen on four mesotrophic lakes. Heming L. and Quigly L. (54°53'N, 101°07'W) are two small (<220 ha), adjacent lakes; Quigly L. has T. crassus whereas Heming L. does not. Falcon L. (49°42'N, 95°18'W) is a moderate-sized (1600 ha) lake. Southern Indian L. (56°47'N, 98°54'W) is a large (225,000 ha) lake: the Long Bay site has still water and a narrow access to the rest of the lake; the Channel site connects two main lake basins and has high current flow. All sites except the Channel are ice-covered in winter. Five collection periods were chosen to examine seasonal temporal variability: midwinter (March); spring (April), when open water was present near-shore only, and pike came into the shallows to spawn; and three summer collections (June, July, and August). Collections at some sites were made in two or four consecutive years to evaluate non-seasonal temporal variability.

COLLECTION OF FISH AND PARASITES

Pike were collected by angling or gill-netting; when gill-netted they were not used if dead or moribund. The pike were killed by a blow to the head and their intestinal tracts were removed and quick-frozen in an ethanol-dry ice mixture (ca. -70C) within 2 min of death. This minimized post-mortem parasite movements that might produce artifacts of their spatial distribution in the intestine.

Parasites were recovered from intestines by two procedures. The first procedure was applied to quick-frozen intestines and to a sample of fresh intestines from Falcon L. pike (the latter were examined within 1 h of host death to recover T. crassus for fecundity assessment and will be described in Chapter 3; air temperature was 0-5C during these examinations and post-mortem worm movement was not observed). The intestine was slit longitudinally and pins inserted to subdivide the intestine into 20 equal, 5% sections of total length between the pylorus (0% position) and the intestinal-rectal valve (100% position). The rectum was considered to be section 21 (105% position). The bodies of all worms were moved to the section in which their anterior ends were attached, and the sections were then cut for separate examination. Examination of each section involved a census of each species, and categorization of each worm by sex and state of maturity. This procedure permitted reconstruction of the spatial distribution of attachment sites. Helminths, grouped by species, were placed on glass slides, dried at 70C for 48 h, then weighed to the nearest 0.01 mg.

The second procedure was applied to a random sample of pike collected at the Channel. Intestines were cut into 20 equal, 5% sections of total length before being opened longitudinally in order to minimize disturbance of the linear spatial arrangement of worm bodies. Each section was examined separately and the number of individuals of each species attached in each section was recorded (all species could be identified by anterior body morphology). This procedure provided comparable data on attachment site distribution to that provided by the first procedure. The mass of nematodes and acanthocephalans was determined as above. Segments of cestode strobilae, which were identifiable to genus, were sorted by genus and section of occurrence and mass determinations done as above. Following worm recovery by both procedures, the lumenal surface of the intestine was examined for signs of pathology (areas with pits or missing epithelium) that might alter suitability for parasite attachment.

Mature Triaenophorus spp. detach from the intestine and release all their eggs upon contact of their strobilae with fresh water (Kuperman 1973). Fecundity of T. crassus (which will be described in more detail in chapter 3) was assessed by removing live worms from freshly-killed pike at Falcon L., placing them in separate test tubes, and covering them with dechlorinated water. After 20 h the number of eggs released into the water was estimated using serial dilutions. The uteri of spent worms were empty or retained less than ca. 5% of the eggs.

Cross-sectional areas of the lumen were determined using a

planimeter on photographs of cut sections on a sample of five previously-frozen intestines, and from drawings of histological sections.

A conversion factor was determined to estimate helminth volume from mass. From each of five pike all helminths were removed, the total volume of water they displaced in a 10-ml graduated cylinder measured, and then total mass determined following drying.

Stomach contents of all pike were identified and a subsample of cisco (Coregonus artedii) from pike stomachs was examined for larval T. crassus.

DATA ANALYSIS

Terminology for prevalence, intensity, abundance, mean intensity, density, and infrapopulation follows the definitions in Margolis et al. (1982). Prevalence was the proportion of pike infected, and intensity was the number of parasites of a given species in an infected pike. The infrapopulation of a species comprised all individuals of that species within a single pike. The frequency distribution of intensities was tested for fit to a negative binomial distribution, using the method of maximum likelihood, for each species in each of the 17 collections of pike. The shape parameter k of the distribution was used as an intrinsic measure of the degree of overdispersion of each species among pike.

Several measures of attachment site and mass location of each species within the intestine of each pike were used: (1) the median % position, calculated using the method for grouped data (Sokal and Rohlf 1981), (2) the number of sections occupied (out of 21) by at least one attachment site, (3) the most anterior (low % position) and most posterior (high % position) location of attachment sites or mass. In addition, a fundamental distribution of attachment sites and mass for each species was derived by taking the sum of attachment sites (or mass) in each section, over all pike examined. Multimodality of attachment site distributions within infrapopulations of T. crassus was quantified. Modes were operationally defined as sections having peak numbers of attachment sites that were separated from other such peaks by at least one section having fewer than one-half the number of sites of the smaller peak; sections with fewer than five sites were not considered to be modal.

Setwise multiple regression (Tabachnick and Fidell 1983), using the maximum R^2 improvement technique, assessed the magnitude of correlation between several independent variables (the intensities and mass per worm of eight species recovered in this study; intestine length of host; extent of pathology in host intestine) and two types of dependent variables: the median % position of attachment by each infrapopulation; the number of 5% sections of intestine in which at least one individual of a given species was attached. Following use of logarithmic transformations on all independent and some dependent variables, visual examination of residual plots indicated the relationships were linear, with residuals independently and normally distributed about the regression lines. The full models included up

to 18 independent variables, but models incorporating a smaller number of independent variables were selected using the Cp statistic (Daniel and Wood 1980).

Monte Carlo randomization techniques were used to test the null hypothesis that the ordering of median % positions of each species along the length of the intestine could be explained by the attachment of each species independently from the location of other species. Determination of the independent attachment site preference of each species (i.e. in the absence of other species) was not possible since all but two pike had multispecies infections. The pool of median % positions from all hosts sampled was used as the best available estimate of each species' independent preference since it defined a range which could be occupied and varying probabilities of occupation within that range. For each particular n- species community that was observed in x pike, an observed frequency distribution for the n! possible orderings of species was constructed. An expected frequency distribution under the null hypothesis was derived by randomly assembling x, n -species communities from the appropriate pools of median positions. Fifty expected frequency distributions were derived, and the mean and standard deviation ($\bar{X} \pm SD$) of each of the n! orderings was calculated. Observed orderings occurring more than 2 SD above or below the expected mean number were considered to be significant departures from the null hypothesis.

Overlap in spatial distribution between species pairs was estimated by an index of percent similarity (Hurlbert 1978): Cxy= 100 min (Pxi, Pyi) where i are resource states and Pxi and Pyi are the

proportions of two species that occur in resource state *i*. The 20 intestinal sections of pike were used as separate resource states. Overlap was calculated for species pairs within each pike and then averaged across all pike (realized overlap) as well as from the summed distributions of each species (fundamental overlap). A Monte Carlo randomization technique was employed to assess whether observed overlaps (percent similarity) between *T. crassus* and *P. pinguis* within a pike could be explained by independent site selection of the two species. This procedure was done on a collection of 41 pike from Southern Indian L., July, 1981, the largest sample with data on both mass and attachment site distributions. One pike was selected at random to provide data on *T. crassus* and another pike was independently and randomly selected to provide data on *P. pinguis* overlap was then calculated. This procedure was done 41 times, and the distribution of overlap values was compared with the distribution observed in those 41 pike using a Wilcoxon two-sample test. Separate trials were done for mass and attachment site overlaps.

Data analysis used the Statistical Analysis System (SAS Institute Inc., Box 8000, Cary, NC, 1982 version), as implemented by the University of Manitoba Computer Services, except for fitting of the negative binomial distributions, Monte Carlo techniques, and calculations of overlap, which used programs written in APL. All data are presented as $\bar{X} \pm$ SD (N) unless otherwise indicated. Statistical significance was determined using $\alpha = 0.05$.

VOUCHER SPECIMENS

The following specimens are deposited in the National Museum of Natural Science, Ottawa, Canada K1A 0M8. NMNS catalogue numbers are: *Triaenophorus crassus*, NMCP1986-0838; *Triaenophorus nodulosus*, NMCP1986-0837; *Proteocephalus pinguis*, NMCP1986-0836; *Raphidascaris acus*, NMCP1986-0840 and 0841; *Neoechinorhynchus tenellus*, NMCP 1986-0842 to 0844; *Leptorhynchoides thecatus*, NMCP1986-0845 and 0846; *Echinorhynchus leidyi*, NMCP1985-0134 to 0139; *Echinorhynchus salmonis*, NMCP1985-0150 to 0153; *Centrovarium lobotes*, NMCP1986-0835.

RESULTS

A total of 366 helminth infracommunities was examined with a minimum of six in each collection (Table 1). The range in fork lengths of the pike was 244-853 mm, but to provide a more equitable size distribution of pike for comparisons between collections the analysis was restricted to pike 300-700 mm fork length (N= 337) when required. The data were evaluated first for more general aspects of the helminth community, such as species abundances, and then for more specific aspects, such as site selection and intraspecific and interspecific interactions.

Eight species of helminth were commonly encountered (Table 1), and three additional species (Pomphorhynchus bulbocolli, Centrovarium lobotes, and Crepidostomum sp.) were present in one or two pike but were excluded from all analyses. Detailed data on parasite numbers is in Appendix I. Triaenophorus nodulosus, Proteocephalus pinquis, and Raphidascaris acus were present in all lakes, T. crassus was present in all but Heming L., Neoechinorhynchus tenellus and Leptorhynchoides thecatus were in Falcon L. only, and Echinorhynchus leidyi and Echinorhynchus salmonis were in Southern Indian L. only. The prevalence of P. pinquis and R. acus was high in all sites while the prevalence of the remaining species varied between sites or seasonally within a site (Table 1). Intensities of all species varied seasonally

Table 1. Prevalences and mean intensities of helminths in 17 collections of northern pike. Pike 300-700 mm fork length were used.

Code[a]	No. pike	Helminth species[b]							
		TC	TN	PP	RA	EL	ES	NT	LT
HJn81	8	0;-[c]	100;14	100;507	100;62	0;-	0;-	0;-	0;-
HJ181	23	0;-	91;17	100;63	78;3	0;-	0;-	0;-	0;-
QJn81	11	18;2	54;4	100;97	100;24	0;-	0;-	0;-	0;-
FAp81	19	84;10	26;3	100;186	63;30	0;-	0;-	37;5	21;4
FAp82	8	82[d];19	29[d];3	100;192	88;15	0;-	0;-	38;1	38;29
FAp83	21	71;13	52;9	100;122	76;19	0;-	0;-	14;1	28;11
FAp84	15	73;16	40;3	100;105	80;13	0;-	0;-	7;1	40;9
LJn81	13	100;66	46;6	100;211	100;38	61;3	8;1	0;-	0;-
LJ181	11	82;24	55;4	100;22	64;6	0;-	0;-	0;-	0;-
LAu81	7	86;27	86;5	100;47	86;6	0;-	0;-	0;-	0;-
CJn81	6	100;47	33;30	100;47	100;17	17;5	0;-	0;-	0;-
CJ181	58	100;102	17;11	100;54	100;35	59;6	14;2	0;-	0;-
CAu81	20	100;91	35;2	100;32	100;29	20;2	20;2	0;-	0;-
CMr82	12	100;78	33;2	100;119	100;33	75;2	8;1	0;-	0;-
CJn82	25	92;50	36;6	100;65	96;19	32;4	4;1	0;-	0;-
CJ182	39	97;71	20;2	97;60	90;17	44;5	20;2	0;-	0;-
CAu82	47	100;70	26;7	98;43	98;23	11;1	2;1	0;-	0;-

[a] Collection site (H, Heming L.; Q, Quigly L.; F, Falcon L.; C, Channel site on Southern Indian L.; L, Long Bay site on SIL); Month; Year.

[b] Cestodes: TC (Triaenophorus crassus); TN (T. nodulosus); PP (Proteocephalus pinguis). Nematodes: RA (Raphidascaris acus). Acanthocephalans: EL (Echinorhynchus leidyi); ES (E. salmonis); NT (Neoechinorhynchus tenellus); LT (Leptorhynchoides thecatus).

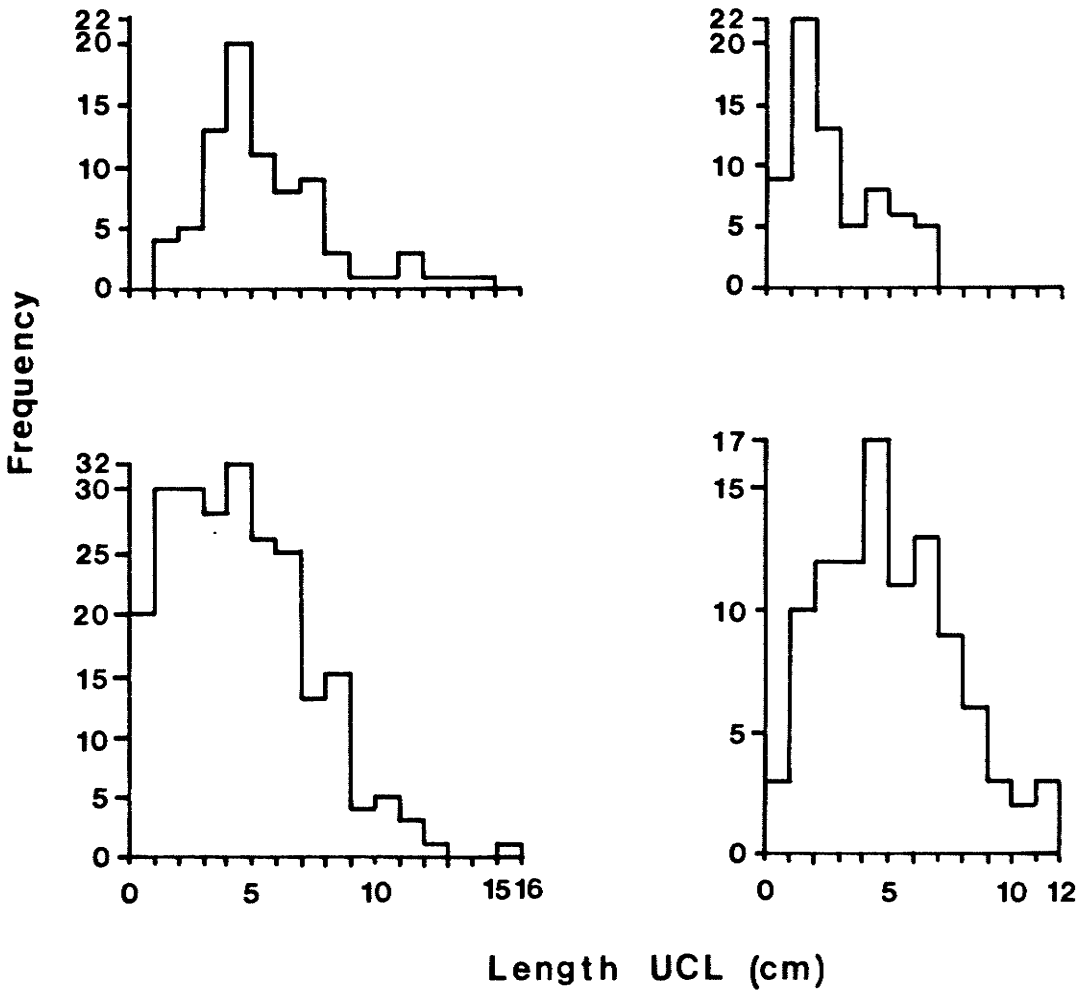
[c] Percent infected; $\bar{X}$ intensity.

[d] Based on 17 pike.

and between sites. Prevalence and intensity of all species was similar between years at the same site and time of year (Table 1).

Sufficient data was available from the Channel site to examine seasonal patterns of recruitment for most species (Table 1). Numbers of T. crassus were lowest in June, increased by July, and thereafter remained steady. Length-frequency distributions of all T. crassus from four pike in a July sample from the Channel were approximately unimodal (Fig. 5) and therefore indicated that larger worms remaining from the previous year's infection were few. Mature T. crassus were observed primarily in March and June. These observations indicate that T. crassus was recruited in early summer, matured in late winter, and passed out of the pike the following spring. Recruitment patterns of T. nodulosus could not be assessed due to low sample sizes, but mature T. nodulosus were found primarily in March and June, similar to T. crassus. Numbers of P. pinguis were greatest in the March collection and declined towards late summer (Table 1) with a corresponding increase in the proportion of mature worms. This suggests a late summer and overwinter recruitment of P. pinguis, with maturation occurring in late spring, followed by loss of mature worms. A strong seasonal pattern in numbers of R. acus was not evident, but over 60% of worms in March and June were mature, while less than 30% were in July and August, suggesting that R. acus matured over winter, with loss of mature worms the following summer associated with recruitment of larval R. acus. The proportion of mature R. acus in the five June collections from northern lakes was 55-65% (X^2= 7.15, df= 4, P= 0.13). The acanthocephalans E. leidyi and E. salmonis generally had higher prevalences and intensities during March and June

Figure 5. Frequency distribution of lengths of individual T. crassus in four northern pike collected in July, 1981, from Southern Indian Lake, Manitoba. UCL, upper class limit.


Frequency
22
20
15
10
5
0
22
20
15
10
5
0
32
30
25
20
15
10
5
0
0
5
10
15 16
17
15
10
5
0
0
5
10 12
Length UCL (cm)

than in July and August, suggesting that recruitment occurred during fall and winter. These interpretations of the phenology of T. nodulosus, P. pinquis, and R. acus from the Channel were supported by limited seasonal data from Heming L. and Long Bay (Table 1), but T. crassus at Long Bay were lost from pike later in the summer and recruitment during the summer was lower than at the Channel.

Infection intensities of T. crassus, T. nodulosus, P. pinquis, and R. acus had negative binomial distributions (the other species were not tested). Linear regression (weighted by the inverse of the variance of k) indicated a significant increase in k relative to mean intensity of T. crassus, a marginally significant increase for R. acus, and no significant dependence on mean intensity for T. nodulosus and P. pinquis (Table 2).

Spearman rank correlations between intensities of all species pairs in each of the 17 collections were used to test for associations between species. Of 197 possible correlations, four were significantly negative and 30 were significantly positive. The most frequently observed positive correlations were between P. pinquis and R. acus (8 of 17 possible), and E. leidyi and E. salmonis (3 of 7 possible). Three of the four July and August collections at the Channel in 1981 and 1982 had significant positive correlations between T. crassus and R. acus.

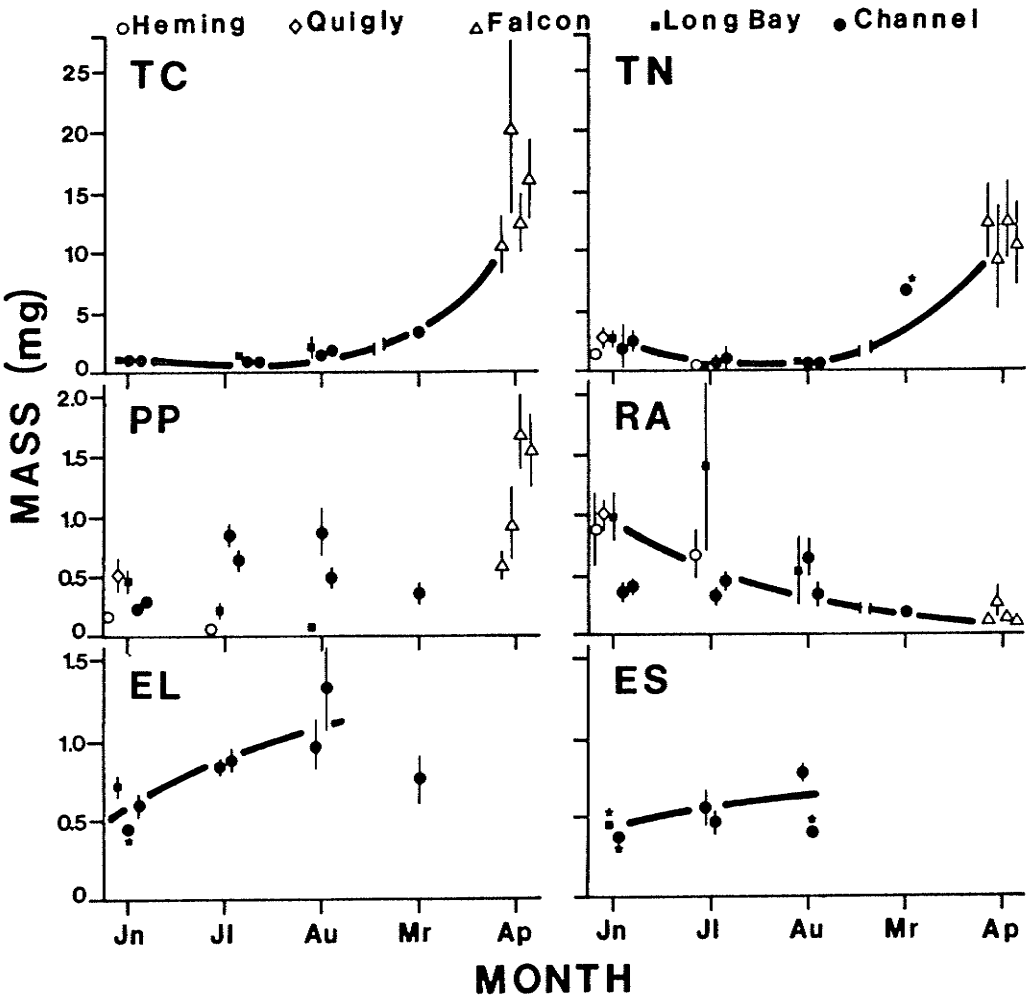
Five species (T. crassus, T. nodulosus, R. acus, E. leidyi, and E. salmonis) exhibited consistent seasonal changes in mean mass, but due to variation between collection sites a general seasonal pattern for P. pinquis could not be determined (Fig. 6). Maximum mean mass was

Table 2. Weighted linear regression of $\underline{k}$ on mean intensity (X) of helminths of northern pike.

Species[a]	Regression equation	SE(b)	H_o: b= 0
TC	$\underline{k}$= -0.032 + 0.035 X	0.0037	P< 0.001
TN	$\underline{k}$= 0.13 + 0.0084 X	0.0082	P= 0.324
PP	$\underline{k}$= 0.69 + 0.00059 X	0.0011	P= 0.581
RA	$\underline{k}$= 0.018 + 0.028 X	0.014	P= 0.058

[a] Species codes are defined in Table 1.

Figure 6. Seasonal changes in mass of helminths in northern pike 300-700 mm fork length. A single mean mass of each species was determined for each pike. Data represent $\bar{X} \pm$ SEM across all pike from each month and location. *, mean from one or two pike; all other points based on three or more pike.


Heming
Quigly
Falcon
Long Bay
Channel
TC
TN
PP
RA
EL
ES
MASS (mg)
MONTH
Jn
Jl
Au
Mr
Ap

observed for T. crassus and T. nodulosus in April, for R. acus in June, and for E. leidyi and E. salmonis in August. The maximum seasonal range in mean mass per worm (with the range in mass of individual worms shown in parentheses) was ca. 14 mg (250) for T. crassus, 11 mg (40) for T. nodulosus, 2 mg (5) for R. acus, 1 mg (1.3) for E. leidyi, and 0.3 mg (0.8) for E. salmonis (Fig. 6).

Net mass production by T. crassus was assessed at two collection sites. The total mass of T. crassus infrapopulations collected at Falcon L. in April frequently exceeded 1000 mg. These infrapopulations were at most one year old, and therefore net production by T. crassus averaged 2.7 $mg \cdot fish^{-1} \cdot day^{-1}$. At the Channel the net increase in mass of T. crassus between July and August, 1981, was 36 mg/fish, or net production of 1.2 $mg \cdot fish^{-1} \cdot day^{-1}$; between August,1981 and March, 1982 a net increase of 191 mg/fish led to an estimated net production by T. crassus of 0.9 $mg \cdot fish^{-1} \cdot day^{-1}$.

Stomach contents of all pike were examined to assess sources for recruitment of larval helminths (Table 3). More than half of the stomachs examined from Long Bay were empty; those with food contained invertebrates (11 of 15 pike) or fish (3 of 15). Pike from other sites were predominantly piscivorous but feeding rate varied seasonally: 27% of stomachs in March-June contained food compared with 62% in July and August. Pike from the Channel fed almost exclusively on cisco (92% of all identified fish in stomach contents). These pike had 0.3 $\pm$ 0.5 (N=11 pike) ciscos per stomach in March, 1.1 $\pm$ 2.0 (29) in June, 0.8 $\pm$ 0.8 (93) in July, and 0.7 $\pm$ 0.8 (65) in August. A sample of 39 ciscos from pike stomachs had 1.3 $\pm$ 1.3 plerocercoid

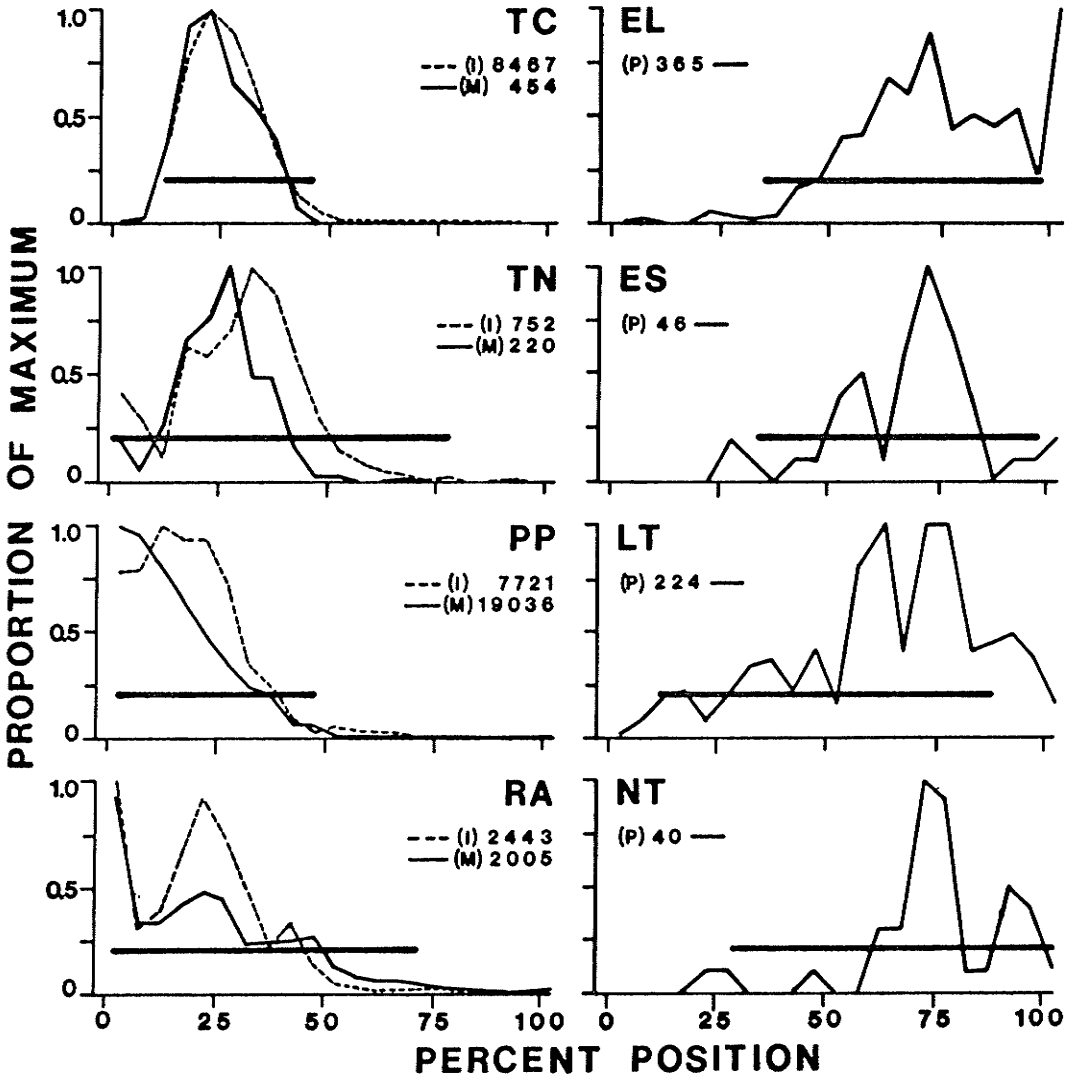
Table 3. Stomach contents of northern pike. Collection site codes are defined in Table 1. Each positive record indicates one pike with that food item present.

	F Ap 81	F Ap 82	F Ap 83	F Ap 84	Q Jn 81	H Jn 81	H Jl 81	L Jn 81	L Jl 81	L Au 81	C Jn 81	C Jl 81	C Au 81	C Mr 82	C Jn 82	C Jl 82	C Au 82
Invertebrates																	
Annelida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Crustacea	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
Insecta	0	0	1	0	1	1	1	7	1	1	2	1	0	0	0	1	0
Total	0	0	1	0	1	1	1	7	4	1	1	1	0	0	1	1	0
Vertebrates																	
Osteichthyes																	
Umbridae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Esocidae	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Coregonidae	0	0	0	0	1	0	0	1	0	2	4	29	10	2	5	15	9
Cyprinidae	1	0	0	0	0	0	2	0	0	0	1	0	0	0	1	0	1
Catostomidae	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Gadidae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Gasterosteidae	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Percopsidae	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0
Percidae	0	0	2	0	1	0	4	0	0	1	0	0	0	0	0	0	0
Unidentified	2	2	2	2	0	3	6	0	0	0	1	4	4	1	3	6	9
Total	4	2	3	2	2	6	13	1	0	2	4	33	14	4	8	22	19
Mammalia	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Total with food	4	2	4	2	3	6	14	8	4	3	5	34	14	4	9	23	19
Total with empty stomach	19	6	22	16	10	2	9	5	7	4	1	19	6	8	16	16	28

larvae of T. crassus each.

Two patterns of fundamental (summed) distributions of helminth attachment sites were observed (Fig. 7): the cestodes and nematodes were in the anterior 50% and the acanthocephalans were in the posterior 50%. Mature and immature T. crassus had similar distributions, but mature T. nodulosus, P. pinquis and R. acus tended to be attached more anteriorly than immatures. Mature male and female R. acus had similar distributions; acanthocephalan numbers were too low to permit detailed inter-sex comparisons but no major distributional differences were noted. The shapes of the summed distributions, calculated separately for each collection of pike, were similar. Mature and immature R. acus had bimodal fundamental distributions (Fig. 7). This pattern was not a pooling artifact since it was evident in each of the 17 collections of pike. Two possible causes of this bimodality, diurnal migration and a response to host feeding activity, were investigated. First, diurnal characteristics of attachment site occupation by R. acus were evaluated on a sample of 57 pike, collected at the Channel over a 48-h period in July, 1981 and at times between 0500-2300 hours. The data were grouped at 3-h intervals and the mean anterior, median, and posterior attachment sites compared. The mean anterior attachment site varied between the 10 and 20% position, the median between 19-35%, and the posterior between 35-60%. The variations did not occur in a cyclic manner corresponding to time of day. Second, the mean locations of worm attachment and mass in feeding and non-feeding pike were determined for each pike collection (where means were available for $N \geq 4$ pike) and these means were compared between collections using a paired t-

Figure 7. Distribution of attachment sites of helminths within the intestine of northern pike. Counts for each 5% section of intestinal length were summed across all pike and then standardized relative to the maximum count per section for each species. The 0 % position is nearest the stomach. The number of individual immature worms (I), mature worms (M), or the pooled number (P) comprising the summed distributions are shown. Horizontal bars indicate the range of median attachment sites within individual fish.


TC
(I) 8467
(M) 454
EL
(P) 365
TN
(I) 752
(M) 220
ES
(P) 46
PP
(I) 7721
(M) 19036
LT
(P) 224
RA
(I) 2443
(M) 2005
NT
(P) 40
PROPORTION OF MAXIMUM
1.0
0.5
0
0
25
50
75
100
PERCENT POSITION

test. The most anterior attachment site of R. acus in feeding pike was on average 7% of intestinal length closer to the stomach, the median was 12%, and the most posterior site was 14% (Table 4). No significant differences in attachment site and mass distributions of T. crassus and P. pinquis were evident between feeding and non-feeding pike (Table 4). The available data would have detected differences >6% intestinal length in anterior and median sites of attachment and mass distribution for T. crassus and P. pinquis, and differences >15% for the posterior sites, assuming that the sample variances would be unchanged (Table 4). Sample sizes for the remaining species were too small for analysis.

The median % positions of infrapopulations of each species varied between pike (Fig. 7), and a one-way analysis of variance was used to test whether median % positions (log transformed) varied between collections of pike. Significant inter-collection differences were not found for T. crassus ($F= 1.21$; $df= 14, 269$; $P= 0.27$; average median position= 25.8%), T. nodulosus ($F= 1.50$; $df= 16, 113$; $P= 0.11$; 25.7%), and E. salmonis ($F= 0.19$; $df= 6, 17$; $P= 0.97$; 65.9%), but were present for P. pinquis ($F= 11.3$; $df= 16, 324$; $P< 0.001$), R. acus ($F= 3.03$; $df= 16, 290$; $P< 0.001$), and E. leidyi ($F= 3.98$; $df= 7, 77$; $P= 0.001$). Gabriel's simultaneous test procedure (Gabriel 1978) was used to test for significantly different pairs of collections for the latter three species. Average median attachment sites of P. pinquis were more anterior in March-June collections, and more posterior in July-August collections (Table 5). Four paired comparisons of average median attachment sites of R. acus differed significantly (Gabriel's test): CJ181 (25% position) and CAu82 (27%) each from CJu81 (9%) and

Table 4. Differences in mean worm locations between pike with and without food in the stomach. Pike 300-700 mm fork length were used.

Species[a]	Location	Mean difference[b]	Significance test[c]	Detection[d]
Attachment				
TC	Anterior	0.03 ± 3.38	t=0.03; df=8; P=0.488	2.6
	Median	-0.48 ± 4.48	t=-0.32; df=8; P=0.379	3.4
	Posterior	1.24 ± 8.72	t=0.43; df=8; P=0.339	6.7
TN	Anterior	4.68 ± 8.38	t=1.11; df=3; P=0.173	13.3
	Median	7.55 ± 8.42	t=1.80; df=3; P=0.085	13.4
	Posterior	9.59 ± 12.5	t=1.54; df=3; P=0.110	19.8
PP	Anterior	-0.25 ± 6.05	t=-0.14; df=10; P=0.446	4.1
	Median	1.02 ± 3.43	t=0.99; df=10; P=0.172	2.3
	Posterior	1.25 ± 13.0	t=0.32; df=10; P=0.378	8.7
RA	Anterior	7.28 ± 4.31	t=5.06; df=8; P< 0.001	3.3
	Median	11.6 ± 10.8	t=3.22; df=8; P=0.006	8.3
	Posterior	13.6 ± 17.3	t=2.36; df=8; P=0.023	13.3
Mass				
TC	Anterior	-1.60 ± 3.88	t=-0.92; df=4; P=0.205	4.8
	Median	-3.11 ± 4.17	t=-1.66; df=4; P=0.086	5.2
	Posterior	3.47 ± 9.32	t=0.83; df=4; P=0.227	11.6
PP	Anterior	-0.79 ± 4.61	t=-0.38; df=4; P=0.361	5.7
	Median	-1.61 ± 1.61	t=-2.25; df=4; P=0.044	2.0
	Posterior	-1.29 ± 11.5	t=-0.25; df=4; P=0.407	14.3

[a] Codes as in Table 1.

[b] Within each collection site the mean % position in pike without food was subtracted from the mean % position in pike with food. These values are the grand means ± SD across collection sites.

[c] Paired t-test.

[d] Minimum detectable difference in % intestinal length at P< 0.05.

Table 5. Variation in average median % position of attachment by P. pinguis in 17 collections of northern pike. Codes are defined in Table 1. Means joined by a common line do not differ significantly at $P < 0.05$ (Gabriel's test).

Collection code	C Jn 81	C Mr 82	C Jn 82	L Jn 81	F Ap 83	F Ap 82	H Jn 81	F Ap 81	L Au 81	Q Jn 81	H Jl 81	C Jl 82	C Au 82	F Ap 84	L Jl 81	C Au 81	C Jl 81
% position	8	9	10	12	13	14	14	14	15	17	17	17	19	19	20	22	22

CJl82 (14%), but with no apparent pattern related to collection site, month, or year. Average median attachment sites of E. leidyi were increasingly posteriad from March through to August, and three paired comparisons differed significantly (Gabriel's test): CAu81 (93%) from CJn82(64%); CJl81 (81%) from CJn82 and CMr82 (each at 62%).

The suite of independent variables used in the multiple regression analysis provided low explanatory power (low R^2) for median attachment sites within pike but showed significant relationships for four species (Table 6). The independent variables provided high explanatory power for the number of intestinal sections occupied by at least one attachment site of a particular species (Table 7). A species' own numbers explained a majority of the total R^2 for the number of sections occupied by that species, as indicated by a large semipartial r^2 for intensity of each species (Table 7). At corresponding intensities T. crassus occupied about half the number of sections that other species did. Increased intensity of all species was associated with a spread in attachment sites in both directions from the median, but for T. crassus, T. nodulosus, P. pinquis and R. acus the anterior spread did not extend into the stomach and posterior spread rarely occurred beyond section 10.

Pathological damage to the pike intestine by attachment of T. crassus increased proportional to the density of T. crassus per section (Fig. 8). Although the most common observation was that of T. crassus attached within pits in the midst of an area lacking epithelium, observations of multimodal distributions were also made where T. crassus were attached around the periphery of areas that had

Table 6. Results of multiple regression to predict the median attachment site of helminths in the intestine of northern pike. Species codes are defined in Table 1. Subscripts of codes are: M, median % position; I, ln(X+1) intensity; W, ln(X+1) mass (mg). A, ln(X+1) area of intestinal mucosa lacking epithelium (mm^2); L, intestinal length (mm).

ANOVA results

TC_M: F= 0.87; df= 18, 199; P= 0.619; R^2= 0.07

TN_M: F= 4.95; df= 4, 73; P= 0.001; R^2= 0.21

PP_M: F= 14.8; df= 3, 252; P< 0.001; R^2= 0.15

RA_M: F= 4.28; df= 2, 230; P= 0.015; R^2= 0.04

EL_M: F= 6.61; df= 7, 59: P< 0.001; R^2= 0.44

Regression equations

$$TN_M = 32 - 6.8\ LT_I - 8.9\ TC_W + 19\ PP_W - 42\ ES_W$$

$$PP_M = 20 - 1.1\ PP_I + 8.1\ PP_W - 2.2\ TC_W$$

$$RA_M = 28 - 2.1\ RA_I + 6.7\ PP_W$$

$$EL_M = 200 - 2.9\ PP_I + 4.6\ RA_I + 13\ TC_W + 21\ PP_W + 18\ RA_W - 29\ L + 2.4\ A$$

Table 7. Results of multiple regression to predict dispersion of attachment sites of helminths in the intestine of northern pike. Species codes are in Table 1. Subscript S is ln(number of 5% intestinal sections with at least one individual attached); other subscripts are defined in Table 6. The semipartial r^2 (sr^2) for intensity of the species of the dependent variable is indicated.

ANOVA results

TC_S: F= 44.1; df= 3, 217; P< 0.001; R^2= 0.38; sr^2= 0.35

TN_S: F= 158; df= 2, 75; P< 0.001; R^2= 0.81; sr^2= 0.63

PP_S: F= 259; df= 2, 253; P< 0.001; R^2= 0.67; sr^2= 0.63

RA_S: F= 66.7; df= 5, 227; P< 0.001; R^2= 0.60; sr^2= 0.38

EL_S: F= 135; df= 2, 64; P< 0.001; R^2= 0.81; sr^2= 0.80

Regression equations

$$TC_S = -0.32 + 0.34\ TC_I - 0.079\ TN_I + 0.069\ PP_I$$

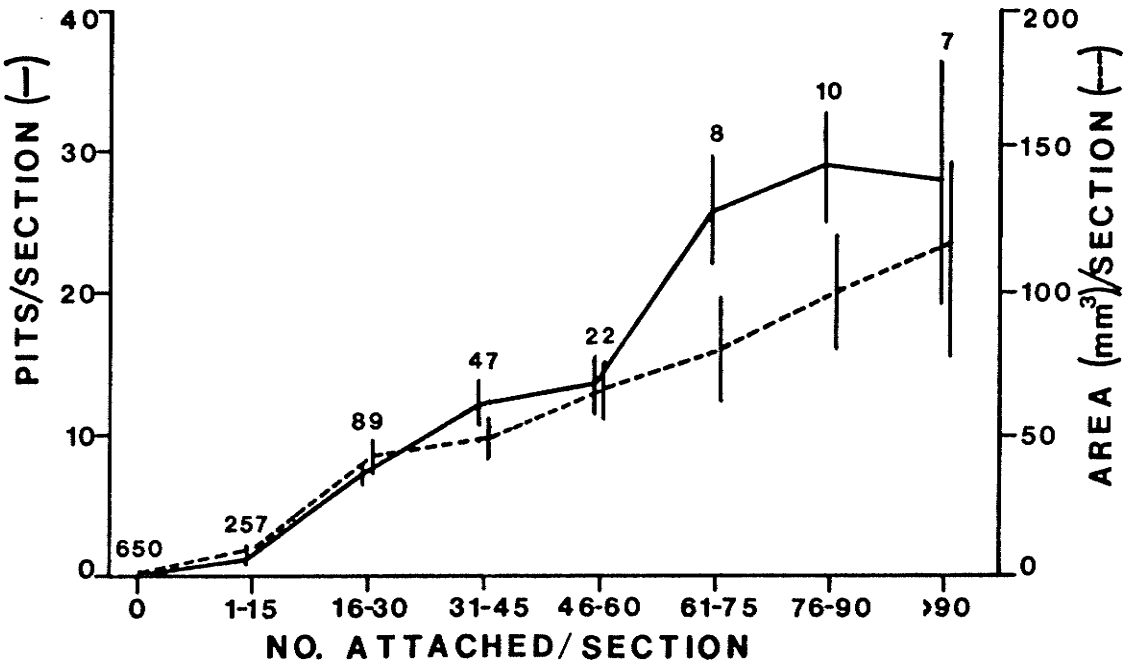
$$TN_S = -0.011 + 0.59\ TN_I - 0.15\ TC_W$$

$$PP_S = 2.4 + 0.35\ PP_I - 0.32\ L$$

$$RA_S = -2.7 + 0.42\ RA_I - 0.96\ TN_I + 0.13\ PP_I + 0.15\ TN_W + 0.42\ L$$

$$EL_S = -0.28 + 0.86\ EL_I - 0.35\ EL_W$$

Figure 8. Pathology in the intestine of northern pike relative to density of attachment sites of T. crassus. Each section is 5% of intestinal length. Pits were depressions around scoleces of T. crassus. Area was lumenal surface of intestine in which epithelium was lacking. Points are $\bar{X} \pm$ SEM (No. sections).


PITS/SECTION (—)
AREA (mm³)/SECTION (—)
40
30
20
10
0
200
150
100
50
0
650
257
89
47
22
8
10
7
0
1-15
16-30
31-45
46-60
61-75
76-90
>90
NO. ATTACHED/SECTION

no epithelium.

A majority of T. crassus infrapopulations at the Channel had a unimodal distribution of attachment sites (Table 8); these modes were in intestinal sections 3-9 (median= 5-6) and did not differ between months (X^2= 9.74; df= 6; P= 0.14). In bimodal infrapopulations the anterior mode was in sections 1-7 (median= 4) and the posterior mode in sections 5-11 (median= 7) (Table 8). The location of modes in bimodal populations did not differ between months (X^2= 0.83; df=4; P=0.93). In trimodal infrapopulations (observed only in July and August) the anterior and middle modes corresponded in position to the two modes of bimodal populations, but the third mode was in sections 7-11 (median= 10) (Table 8). Intermodal regions were frequently characterized by extensive pathology relative to the number of T. crassus attached there.

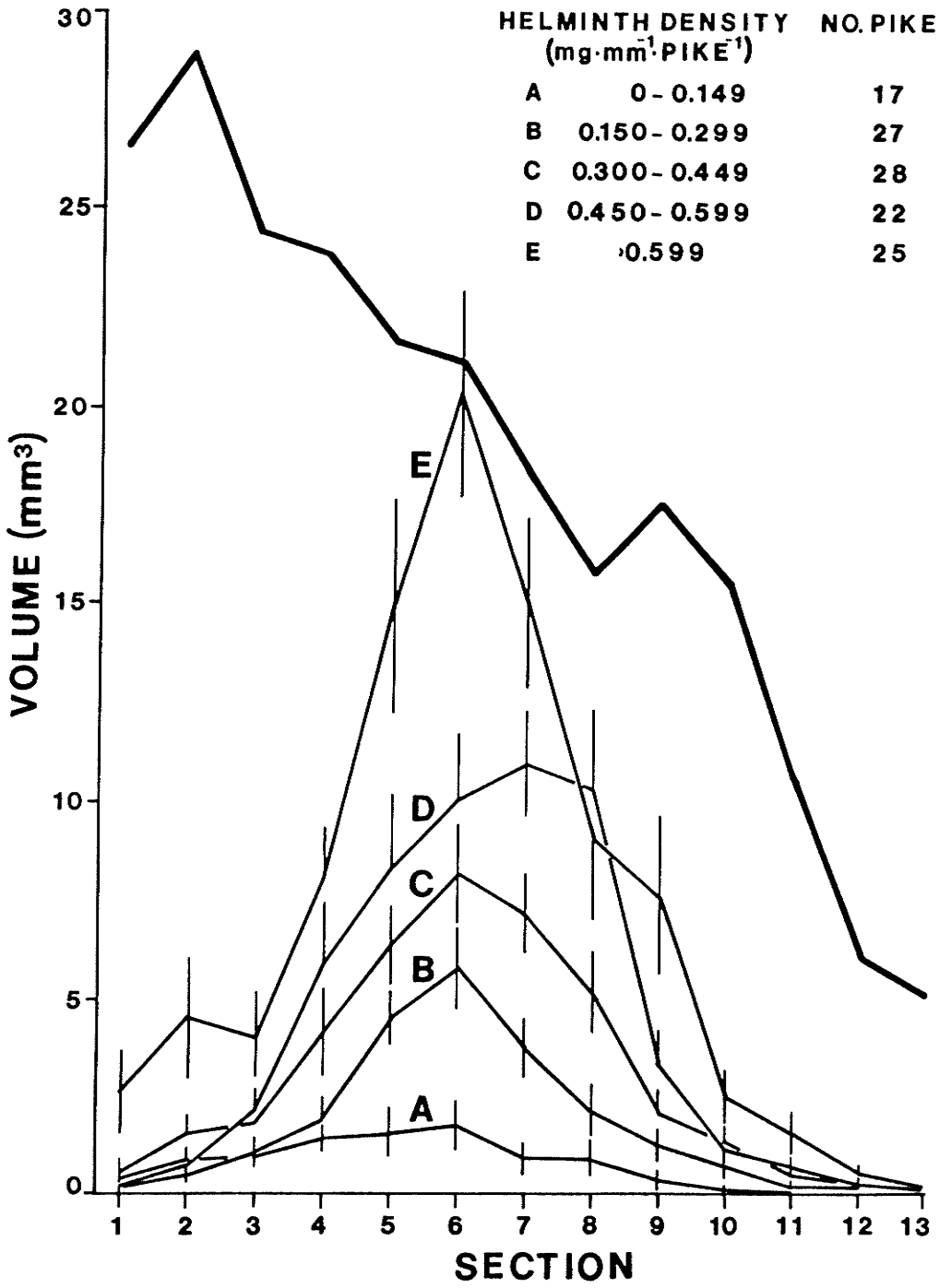
Space occupied by helminths within the intestine was assessed using volume estimated from helminth mass. Measured ratios were 5.6 $\pm$ 0.56 mm^3/mg (N= 5) and mass was converted to volume in Fig. 9 by multiplying by that mean. Pike were classed by overall density of mass in the intestine ($mg \cdot mm \text{ of intestine}^{-1} \cdot pike^{-1}$) to standardize for differences in intestinal length, and within each class the volume occupied by helminths (mm^3/mm of length) was estimated for each section (Fig. 9). As overall density of helminth mass in the pike increased from class A to class D (Fig. 9), there was a greater increase in volume occupied in the posterior sections (5-13) than in the anterior four. By contrast, the additional volume of class E pike was associated with greater increases in the anterior seven sections

Table 8. Location of attachment site modes of T. crassus from uni- and multi-modal infrapopulations in northern pike at the Channel site.

Distribution	Location of mode	Section no. in which modes occur		
		June	July	August
Unimodal	N/A	3-5-8 (22)[a]	3-5-8 (60)	3-6-9 (49)
Bimodal	Anterior	1-3.5-5 (7)	2-3-7 (31)	2-4-6 (14)
	Posterior	6-7-9 (7)	5-7-11 (31)	5-7.5-10 (14)
Trimodal	Anterior	- (0)	2-3-6 (5)	3-4.5-5 (4)
	Median	- (0)	4-7-8 (5)	6-6.5-7 (4)
	Posterior	- (0)	7-10-11 (5)	9-10-11 (4)

[a]Most anterior - median - most posterior (No. fish examined).

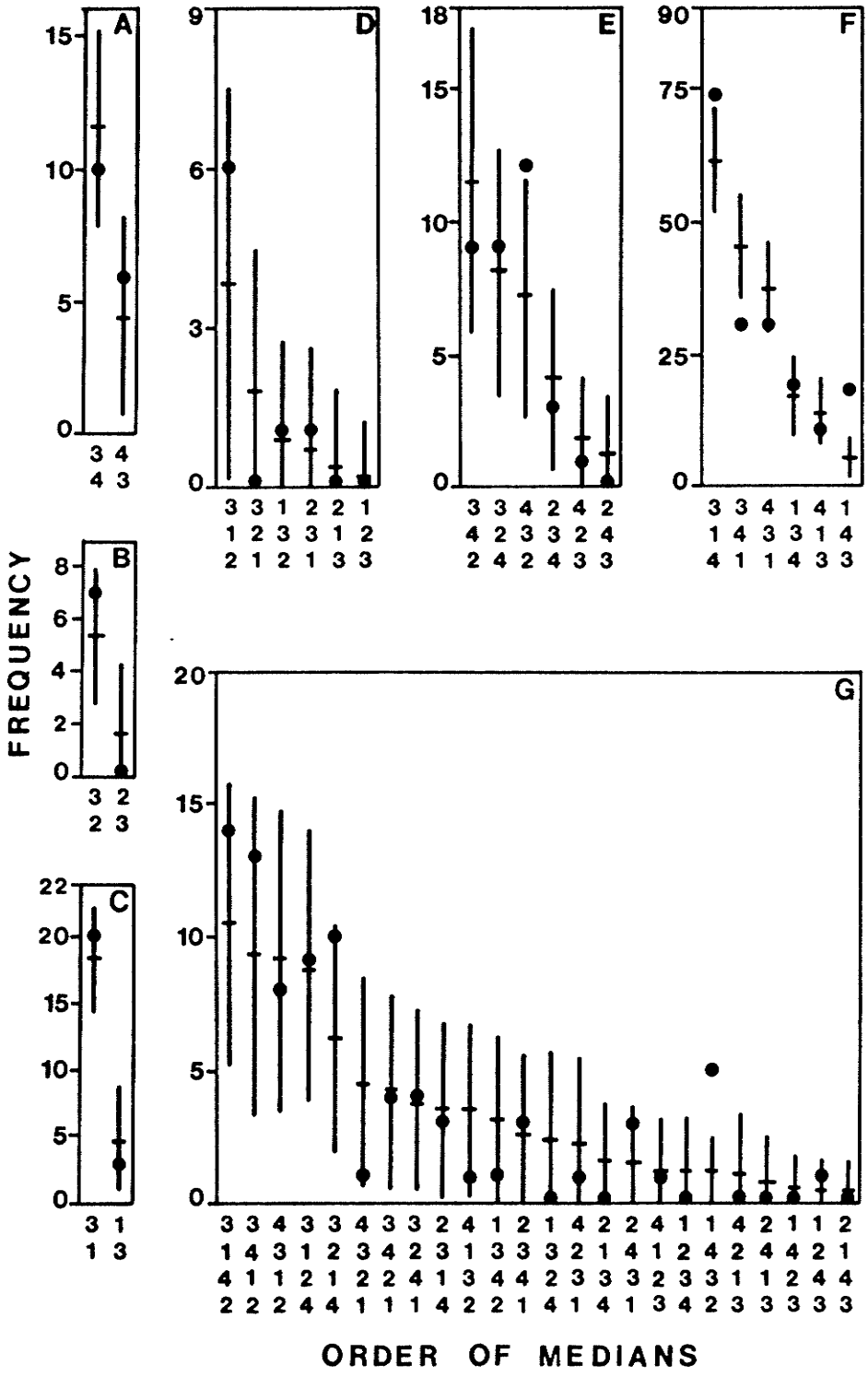
Figure 9. Estimates of lumen volume (thick line) and total helminth volumes (thin lines joining $\bar{X} \pm$ SEM) per mm of intestine in northern pike. Pike were grouped by average density of helminth biomass in their intestine. Each section represents 5% of intestinal length. Sections 14-21 had < 0.01 mm^3 and are not illustrated.


HELMINTH DENSITY (mg·mm⁻¹·PIKE⁻¹)
NO. PIKE
A 0 - 0.149 17
B 0.150 - 0.299 27
C 0.300 - 0.449 28
D 0.450 - 0.599 22
E >0.599 25
VOLUME (mm³)
SECTION
E
D
C
B
A

than in the more posterior sections (Fig. 9). Estimated lumen volume decreased gradually from the stomach towards the rectum (Fig. 9). In the densest infections helminths occupied most of the available space in sections 6-8, but considerable space remained on both sides, particularly anteriad (Fig. 9).

The ordering of infrapopulation medians along the length of the intestine was evaluated only for the four species (T. crassus, T. nodulosus, P. pinquis, and R. acus) which occupied the anterior half of the intestine. Seven of the 16 possible multispecies assemblages occurred (Fig. 10). The null hypothesis that all orderings were equally probable was rejected for four of the five assemblages where sample sizes were sufficient for a X^2 test (Fig. 10) and I concluded that there were interspecific differences in median attachment sites. Using Monte Carlo randomizations, 43 of 48 orderings were observed at a frequency within 2 SD of a frequency dependent only on species-specific site selection characteristics (Fig. 10). Only five orderings (Fig. 10E-G) were more than 2 SD from their predicted frequencies, suggesting a probability of chance occurrence less than 5%. Four of these five orderings involved adjacent infrapopulations of T. crassus and R. acus where the position of T. crassus was anteriad to R. acus more frequently than expected, or R. acus was anteriad to T. crassus less frequently than expected (Fig. 10F,G). However, examination of the collection sites from which pike harboring these unusual orderings occurred, or the maturity of the worms involved, did not reveal any patterns that would suggest any biological significance for this observation. I concluded that observed median locations could still reflect chance establishment of

Figure 10. Ordering of median attachment sites of helminths along the intestine of northern pike. Each order is a 4-digit code where the top digit is the species with the most anterior median, and the bottom digit is the species with the most posterior median: T. crassus = 1, T. nodulosus = 2, P. pinquis = 3, and R. acus = 4. Frequencies predicted from Monte Carlo simulations are shown by horizontal line (mean), and vertical line (± 2 SD). Observed frequencies are shown by circles. X^2 test of H_0 {all orders equally probable}: A, P= 0.600; C, P= 0.002; E, P= 0.001; F,G, P< 0.001; B,D, not tested.


A
B
C
D
E
F
G
FREQUENCY
ORDER OF MEDIANS

medians by each species independent of the location of medians of other species.

Overlaps of attachment sites for all species pairs are shown in Table 9. Fundamental overlaps (using summed distributions) were 56-78% between the four species characteristic of the anterior intestine (T. crassus, T. nodulosus, P. pinguis, and R. acus), 59-74% between the species of the posterior intestine (E. leidyi, E. salmonis, N. tenellus, and L. thecatus), and 7-29% between members of the two groups. Realized overlaps (within individual pike) averaged 22-49% for the anterior species, 9-16% for the posterior species, and 0-10% between members of the two groups. Realized overlaps generally increased in proportion to the sample sizes of the two species, but there were few cases in which several species occurred in large numbers within the same pike. For example, only 18% of the 366 pike helminth communities had two or more species each represented by $N \geq 50$ individuals, 3% with three or more, and none with four or more. The fundamental overlap of T. crassus and P. pinguis mass was 82%; realized overlaps were $40 \pm 21.1\%$ (119).

Regression coefficients were examined in all significant regressions (Tables 6 and 7) to assess relative contributions of intraspecific and interspecific factors to attachment site selection by the five commonest helminth species. The median % positions of P. pinguis and R. acus varied with their own mass or intensity as well as with those of other species (Table 6). Interestingly, the intensity or mass of T. nodulosus and E. leidyi did not contribute to explaining their own median position, but E. leidyi was found more posteriad when

Table 9. Pairwise comparisons of percent overlap of helminth attachment sites in the intestine of northern pike. Upper right of trellis, overlaps of summed species distributions; lower left, overlaps calculated for each host, then averaged.

Species[a]:	TC	TN	PP	RA	EL	ES	LT	NT
TC	X	71	56	71	7	11	22	7
TN	22±26[b] (88)	X	60	78	13	15	29	9
PP	25±19 (282)	23±18 (128)	X	72	7	11	22	7
RA	49±29 (258)	22±25 (113)	32±22 (306)	X	14	16	29	10
EL	1±8 (85)	2±10 (21)	1±2 (85)	2±7 (84)	X	70	74	60
ES	2±10 (24)	0±0 (6)	1±4 (24)	5±15 (24)	9±15 (18)	X	63	61
LT	10±17 (11)	10±21 (11)	10±13 (19)	8±10 (18)	-[c]	-	X	59
NT	0±0 (13)	0±0 (6)	4±12 (14)	6±12 (10)	-	-	16±20 (5)	X

[a] Species codes are in Table 1.

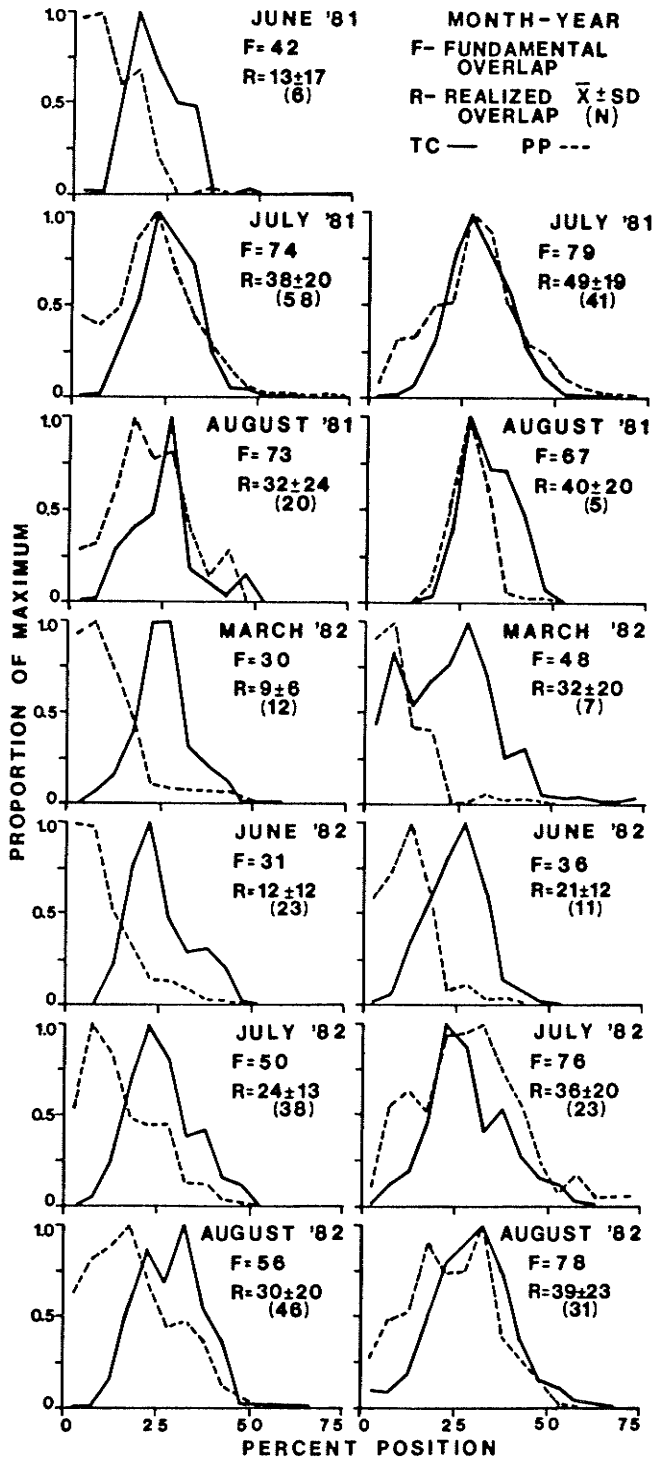
[b] $\bar{X} \pm$ SD (no. hosts with both species)

[c] Species did not co-occur.

there was pathology caused by T. crassus (Table 6). None of the variables had significant explanatory power for the median % position of T. crassus, and it is noted in particular that median % positions of all species except E. leidyi were not significantly related to intestinal length. The dispersion of attachment sites as measured by the number of sections occupied was positively related to each species' own intensity but the relationship to intensity and mass of other species was variable (Table 7).

Several possible cases of negative interspecific interactions were suggested by the regression analyses in Tables 6 and 7, but only one pair of species, T. crassus and P. pinquis, was selected for more detailed analysis since these two cestodes commonly co-occurred in large numbers, both acquire nutrients by absorption, and detailed data on their attachment site and mass distributions was available from a single collection site over two years. The fundamental attachment site distributions of T. crassus over seven consecutive collections at the Channel were similar (Fig. 11) and it was demonstrated earlier that median % positions of T. crassus did not vary significantly between collections. By contrast, the fundamental distributions of P. pinquis (Fig. 11), and the medians in individual fish (presented previously), varied seasonally. Overlaps of fundamental attachment site distributions of the two species were slightly lower in March and June than in July and August (30-42% vs. 50-74%) but realized overlaps were much lower (8-13% vs. 24-38%). The mass distributions of T. crassus and P. pinquis had high fundamental and realized overlaps in July and August, but lower overlaps in March and June (Fig. 11). The mass of T. crassus was symmetrically distributed about the mean in all

Figure 11. Seasonal changes in the distribution of attachment sites (left column) and mass (right column) of T. crassus (TC) and P. pinquis (PP) within the intestine of northern pike. Axes are defined in Fig. 7. Fundamental percent overlaps were calculated on the summed distributions shown; realized overlaps were calculated for individual pike.


MONTH-YEAR
F- FUNDAMENTAL OVERLAP
R- REALIZED OVERLAP X̄ ± SD (N)
TC —— PP ---
JUNE '81
F=42
R=13±17
(6)
JULY '81
F=74
R=38±20
(58)
JULY '81
F=79
R=49±19
(41)
AUGUST '81
F=73
R=32±24
(20)
AUGUST '81
F=67
R=40±20
(5)
MARCH '82
F=30
R=9±6
(12)
MARCH '82
F=48
R=32±20
(7)
JUNE '82
F=31
R=12±12
(23)
JUNE '82
F=36
R=21±12
(11)
JULY '82
F=50
R=24±13
(38)
JULY '82
F=76
R=36±20
(23)
AUGUST '82
F=56
R=30±20
(46)
AUGUST '82
F=78
R=39±23
(31)
PROPORTION OF MAXIMUM
1.0
0.5
0
0
25
50
75
PERCENT POSITION

samples but one: in March, mass of T. crassus was found throughout the anterior 30% of the intestine, anteriad to the attachment sites, and the mass of P. pinquis at that time was more anteriad than at other times of year (Fig. 11). The patterns of attachment site and mass distribution noted during 1981 were re-established in 1982.

Observed attachment site overlaps between T. crassus and P. pinquis in July, 1981, at Southern Indian L., were 0-68% (median= 38%; N= 41 pike) and overlaps of randomly-assembled pairs were 0-57% (median= 37%; N= 41 randomizations) (Wilcoxon test, $P > 0.40$, NS). Observed mass overlaps between T. crassus and P. pinquis in those same pike were 0-86% (median= 49%; N= 41 pike) and overlaps of randomly-assembled pairs were 0-83% (median= 40%; N= 41 randomizations) (Wilcoxon test, $P < 0.05$).

Each infrapopulation of helminths had an unknown but presumably varied age structure, which prevented definitive assessment of the effects of intraspecific and interspecific interactions on worm growth, maturation, or fecundity. However, the highly synchronized annual life cycle of T. crassus, which begins by recruitment of a new infrapopulation each spring and summer, probably resulted in a relatively uniform age structure by the following spring making a crude assessment feasible. At the Channel in particular there was no net recruitment after July (Table 1) suggesting that the age of most worms the following spring was within 1-2 months. At the Channel, where overwinter intensities of T. crassus were ca. 78/pike (Table 1), 17 of 23 infected pike in March-June had at least one mature T. crassus (maximum = 20), while at Falcon L., with a lower mean

intensity (10-19/pike; Table 1), 36 of 60 infected pike had at least one mature T. crassus (maximum = 29) (X^2= 0.29; df= 1; P= 0.59). The mean mass of mature T. crassus in 36 pike from Falcon L. was not correlated with total number of mature T. crassus in those pike (r= 0.11; t= 0.67; df= 39; P= 0.25) or with the total number of T. crassus (r= 0.081; t= 0.47; df=39; P= 0.32). In infrapopulations of T. crassus at Falcon L. where intensities were 1-30, 108 of 417 T. crassus were mature (with eggs in utero), while at intensities of 31-73, 153 of 384 were mature (X^2= 8.96; df= 1; P= 0.003). Fecundity of mature T. crassus was 0-4,400,000 eggs/worm, and total egg release was 0-15,000,000 eggs/infrapopulation. In pike harboring mature T. crassus intensity and egg release per worm were not correlated (r= 0.12; t= 0.62; df= 26; P= 0.27), but there was a positive correlation between intensity and the total number of eggs released per infrapopulation (r= 0.54; t= 3.14; df= 26; P= 0.009). The efficiency of egg production ranged from 260 to 78,500 eggs/mg dry mass of mature T. crassus but it was not correlated with the total mass of helminths in those pike (r= -0.15; t= -0.66; df=19; P=0.26).

DISCUSSION

A natural experiment was used to study community structure of intestinal helminths in northern pike. This provided a series of static pictures of naturally-occurring communities with which to reconstruct dynamic relationships. There are inherent problems with this approach (Connell 1980) since prior history of each community cannot be known absolutely, nor can cause-and-effect be proven. However, the seasonal nature of parasitic infections in temperate regions lends predictability to the composition of helminth communities throughout the year and therefore approximate knowledge of their past histories as well. This seasonality also approximates the effect of experimental perturbations of helminth communities through naturally occurring sequences of colonization and extinction of the component species at the level of the host individual. Characteristics of the pike helminth community were interpreted against the background of seasonal data that was collected. In addition, by measuring parasite mass and intestinal location, and several host attributes, inferences could be made regarding utilization of nutrient, attachment site, and lumen space resources within the community, which has been speculated on but not quantified in previous studies on naturally occurring helminth communities. This discussion will first consider environmental heterogeneity, then the three resources and the relevant interactions involving each, and

finally their contribution to community structure.

ENVIRONMENTAL HETEROGENEITY

The number of individuals in a population may reflect equilibrial levels determined by resource availability, but heterogeneity of both the physical and biological components of the environment can maintain lower population levels (Caswell 1976; Wiens 1977; Connell 1980; MacNally 1983; Price 1984; Sousa 1984). There was considerable variation of intensities for all helminths among pike within each of the 17 collections and between collections. This resulted from geographic variability in the parasite species available for colonization and from geographic and temporal variability in parasite recruitment. In addition, within each collection, where geographic and temporal effects had been removed, there was overdispersion of parasites among pike which was described by a negative binomial distribution. This overdispersion, common in helminth infections, can result from heterogeneity in a variety of host attributes and in the distribution of infective stages (Crofton 1971; Anderson and Gordon 1982; Arnason et al. 1986). Overdispersion means that most pike harbored low numbers of a given species of parasite and smaller numbers of pike harbored high intensity infections. Overdispersion within each collection, and the varying intensities between collections, suggests that high intensities for any species would be rare and that the probability of a host infected with large numbers of

several species would be low. This was observed, as the communities in most pike had low intensities of all component species and consequently low realized overlaps in spatial utilization relative to fundamental overlaps. Environmental heterogeneity promoted low intensity infections, which resulted in a low frequency of co-occurrence of potential competitors and reduced intensity of interspecific interactions.

RESOURCE 1: NUTRIENTS

Experimental studies (summarized by Read and Simmons [1963] and Crompton et al. [1983]) have shown that growth of cestodes and acanthocephalans in mammalian hosts is influenced by the quantity and quality of host diet. Cestodes and acanthocephalans absorb nutrients from the intestinal contents, and R. acus probably ingests particulate matter (Chitwood and Chitwood 1974). Parasite nutrient requirements could not be measured directly, nor could nutrient availability from the diet of the pike or from endogenous secretions into the intestine, but it was assumed that nutrient availability would be proportional to food intake by the pike and that nutrient deficiency would cause reduced growth of the helminths. In addition, intestinal helminths of fish are known to respond to host starvation by migrating within the intestine (MacKenzie and Gibson 1970).

Pike fed at the highest rate during the summer, and lowest during winter and early spring, which agrees with previous studies (Borgstrom

1970; Diana 1979; Mann 1982). The diet of pike varied geographically, but piscivory was documented in all collections and at all times, suggesting that geographic and temporal variation in the quality of nutrients available for parasite utilization was minimal.

Nutrient limitations on T. crassus might be predicted due to its large mass increases during peak growth periods in late winter (Miller 1943a; Kuperman 1973) and the large numbers often present. This growth requires a high rate of nutrient input but it occurs at a time of year when food consumption by pike is low (Borgstrom 1970; Diana 1979; Mann 1982) and digestion rates are reduced. However, several observations suggest that nutrients did not limit growth or maturation of T. crassus: (1) Mass and fecundity of mature T. crassus at Falcon L. were intensity-independent. These worms were assumed to be of relatively uniform age due to their synchronized life cycle, and if nutrients were limiting worms from high intensity infections would be predicted to be smaller (Read and Simmons 1963) or less fecund (Jones and Tan 1971). (2) Intensity-dependent inhibition of maturation was not detected. (3) Rate of mass production by infrapopulations of T. crassus was higher at Falcon L. over winter than at the Channel during summer, yet food consumption by pike at the Channel was the highest recorded in this study. (4) The anterior, median, and posterior locations of attachment sites and mass of T. crassus and also P. pinquis did not differ significantly with the absence or presence of food in the stomach of the pike, indicating that reduced nutrients did not initiate a migration of T. crassus. Temporary limitations may have occurred but did not have detectable effects.

Unlike the cestodes, the location of R. acus varied with host feeding activity. Nematodes respond to host feeding although migrations of some nematodes occur when nutrients are abundant as well as limiting (Croll 1976). The reason for the preference of larval R. acus for lesions generated by attachment of T. crassus is not clear, but the continuous nature of possible nutrients i.e. sloughed cellular debris and leakage of tissue fluids as discussed in chapter 1 may make it a nutrient-rich site for larvae. If this larval site preference, or the general migration of R. acus relative to host feeding activity, was in response to nutrient limitations then some effects on growth or maturation might be predicted. This does not seem to be the case. Examination of data from the seven June collections of pike, which varied in the types and quantities of food eaten and in the extent of lesions on the intestine, revealed similar proportions of mature and larval R. acus.

RESOURCE 2: ATTACHMENT SITES

A suitable substrate for attachment is essential for non-tissue dwelling parasites in order to prevent dislodgement and expulsion from the host. Part of this substrate includes the mucosal surface of the intestine which varies in structure but to which parasites have adapted by developing attachment organs suitable for these specialized areas (Williams et al. 1970). These attachment organs may elicit an inflammatory reaction that renders the substrate unsuitable for

themselves and other species (Grey and Hayunga 1980; Silver et al. 1980; Ferretti et al. 1984; chapter 1).

The mucosal surfaces of the anterior and posterior halves of the pike intestine are morphologically distinct (Bucke 1971) with cestodes and nematodes attached in the anterior half and acanthocephalans located in the posterior half. Within each half there were interspecific differences in site preference. Evidence for attachment site limitations would be: (1) failure of recruits to establish, indicating the absence of suitable sites, or (2) displacement within the intestine, indicating the utilization of less preferred sites.

Was there evidence that recruits were unable to establish? Larval T. crassus were ingested by pike as they fed on cisco at the Channel throughout the summer of 1981, but while the mean intensity of T. crassus in those pike increased between June and July it did not increase between July and the following March indicating that a carrying capacity had been reached. Additional support was the lower overdispersion (higher k of the negative binomial distribution) in pike from collection sites with higher mean intensities (Anderson and Gordon 1982). This apparent inability of recruits to establish was probably due to intraspecific competition for attachment sites, since at the intensities found (100-200 T. crassus): (1) attachment sites occupied most of the available range as indicated by their fundamental distribution, (2) multimodal infrapopulations were frequently observed in which the intermodal areas were rendered unsuitable for attachment because of pathology, and (3) during the summer other resources, nutrients and space (see section on lumen space), were not thought to

be limiting. Since quantitative measurements of recruitment by the other helminths of pike were unavailable they were not analyzed like T. crassus but the lack of significant relationships between k and mean intensity for the other species suggests that their infrapopulation sizes were not limited.

Was there evidence of displacement within the intestine? A review of the literature found that median location of attachment is generally insensitive to species, sex, or age of host (Kennedy et al. 1976; Butterworth 1982), intensity (Bush and Holmes 1986b), and seasonal or geographic differences (Camp and Huizinga 1980; Muzzall 1980; Kennedy and Lord 1982), but that it may vary moderately with helminth age (Chappell 1969; Cannon and Mettrick 1970; Amin 1975) and to a greater extent with the presence of other species of parasite (Holmes 1962; Chappell 1969; Amin 1975; Riley and Owen 1975; Grey and Hayunga 1980; Silver et al. 1980; Kennedy 1985). Most species expand their range within the intestine as intensities increase (Amin 1975; Hair and Holmes 1975; Butterworth 1982; Kennedy and Lord 1982; Bush and Holmes 1986b) indicating that local intraspecific limitations also occur. Locations of attachment by helminths within pike varied moderately with the presence of other species, but location was primarily related to intraspecific factors such as a tendency to aggregate. Infrapopulation medians occurred at most intestinal locations within the physiological limits suggested by the fundamental attachment site distributions, but regardless of where a median occurred attachment sites were aggregated around it. The site chosen by the initial colonists therefore appears unpredictable, but later colonists apparently responded to some mechanism for intraspecific

aggregation. Similar observations for helminths in eels were made by Kennedy (1985).

Interspecific interactions for attachment sites were minor: (1) The lesions created by T. crassus provided a microhabitat that was clearly preferred by larval but not adult R. acus. Occupation of these lesions would maintain the intestinal position of the larvae but probably would not affect their numbers as large populations of R. acus are known from lakes where T. crassus is absent (Poole 1985). (2) Loss of mucosa around the attachment sites of T. crassus made those areas unsuitable for attachment by P. pinquis as shown in chapter 1, but these unsuitable areas usually comprised only a small portion of the intestinal length that P. pinquis is capable of utilizing. (3) The location of E. leidyi within the posterior half of the intestine was negatively correlated with the variables measured on species occupying the anterior half, but direct interactions were unlikely since there were low overlaps in fundamental distributions. One of the significant variables was the extent of pathology induced by T. crassus, and it is interesting to speculate that this pathology may, through a generalized inflammation of surrounding tissues, render them unsuitable for attachment by E. leidyi.

RESOURCE 3: LUMEN SPACE

Intestinal helminths require space to accomodate somatic growth but lumen volume is finite. Limited lumen space has been suggested to

cause reduced growth of worms and worm loss (Silver et al. 1980; Keymer 1982a; Granath and Esch 1983) but characteristics of space utilization by intestinal helminths have not been quantified. Lumen space in pike was greatest near the stomach then decreased as the intestine tapered towards the rectum. Lumen volume can increase through stretching but connective tissue deposition around scoleces of T. crassus described in chapter 1 probably reduces this capability. Regardless of their densities, parasites of pike generally preferred the second quarter of the intestine. Strobilae of T. crassus were often found anterior to their attachment sites, similar to the behavior of other cestodes (Cannon and Mettrick 1970; McKinnon and Featherston 1982), indicating that the first quarter could be utilized. But the first quarter was substantially occupied only when the preferred second quarter appeared unable to accomodate further increases in parasite mass. The first quarter may be poorer quality space due to the presence of undigested food items that could damage cestode strobilae (Williams et al. 1970).

The cestodes T. crassus and P. pinquis, due to their size and numbers, made up most of the parasite volume. There was evidence for negative interactions between them based on limited space availabilty. Triaenophorus crassus attached in the same intestinal region all year and its mass was located near its attachment sites for most of the year except during rapid growth in late winter; then more anterior locations were occupied. In summer there was high overlap between T. crassus and P. pinquis but in late winter and early spring P. pinquis was located more anteriad. This does not reflect an intrinsic, seasonal response by P. pinquis since the location of P. pinquis did

not vary significantly between June and July at Heming L., where T. crassus was absent, or at Long Bay, where intensity of T. crassus was low, but did at the Channel, where intensity of T. crassus was high. This strongly supports the interpretation that P. pinquis at the Channel were temporarily displaced anteriad by strobilae of T. crassus, which also moved anteriad when their mass increased and lumen space was limited.

COMMUNITY STRUCTURE

Structure of the pike helminth community was evident in four areas: species composition, sequence of colonization and extinction, spatial relationships among species within the intestine, and the presence of interspecific interactions. (1) Triaenophorus nodulosus, P. pinquis, R. acus, and to a lesser extent T. crassus, formed a deterministic group of species in pike from Manitoba lakes as indicated by their high prevalence and intensity in this study and others (Lubinsky and Loch 1979; Poole 1985); trematodes and acanthocephalans were a more stochastic component. (2) The composition of the pike helminth community was not static, since colonization and extinction events were common, but these were predictable from the seasonal patterns of infection, maturation, and death of the component species. Seasonality is an important regulator of parasite numbers in temperate regions and is associated with varying degrees of synchronization of life cycles: T. crassus represents an extreme case where each

infrapopulation matures in synchrony and releases eggs only during the brief spawning period of the pike (Miller 1943a; Kuperman 1973). The length-frequency plots for T. crassus from several pike supported the interpretation that there was little or no carryover of individuals from one year to the next as was suggested by Miller (1943a). (3) The spatial distribution of helminths within the intestine is frequently cited as evidence for community structure (Holmes 1973; Price 1980). The helminths of pike segregated into species of the anterior and posterior intestine. For the more common, anterior species, a detailed evaluation of site selection indicated that there was non-random ordering of their median attachment sites. Several orderings were common, and several were not observed. However, using a Monte Carlo simulation it was shown that this was consistent with a collection of species locating independently within the range of the intestine to which they are adapted. The wide overlap in fundamental distributions of these species, but the small portion of each species' range that was usually occupied due to low intensities and a tendency for intraspecific aggregation, explains the large number of different orderings of median attachment sites that was observed. Moreover, realized overlaps of attachment sites and mass between T. crassus and P. pinguis were low, but Monte Carlo randomizations suggested that this could occur without invoking interspecific interactions. (4) Several interactions, defined as the active or passive modification of access to resources by one species on a second (MacNally 1983), occurred within pike. Triaenophorus crassus reduced lumen space availability for P. pinguis, elicited a host response which enhanced attachment site and possibly nutrient availability for larval R. acus,

and perhaps indirectly reduced attachment site suitability for E. leidyi. Interactions are also suggested by correlations in numbers of independently colonizing species (Seifert and Seifert 1979) but the correlations observed for helminths of pike may have other causes. The small number of significant negative correlations may be attributable to the 5% type I error that was allowed, and the larger number of significant positive correlations likely indicated that pike ingesting more food items were likely to acquire larger numbers of all parasites.

It was noted earlier that various sources of environmental heterogeneity resulted in communities with small numbers of individuals. Consequently, there was limited interspecific contact within most pike. Within the range of intensities in the pike examined it was concluded that resources were generally not limiting. Intraspecific competition for limited attachment sites by T. crassus was detected, but evidence that other intraspecific or interspecific interactions that were observed caused depressive competition (an effect on a species' survival status such as mortality or reduced growth or fecundity [MacNally 1983]) was lacking. Although interactions rarely occurred, their magnitude was probably intensity-dependent, and it could be speculated that higher intensities than those observed might lead to depressive competition in natural situations. However, these high intensities are probably rare in nature since the range of intensities of the parasites in this study spanned the ranges observed in other studies (Borgstrom 1970; Kuperman 1973; Watson 1977; Poole 1985). Consequently, it is concluded that interactions were a minor contributor to the structure of the pike

helminth community, which in general was non-interactive.

While most ecologists recognize that many communities of both free-living and parasitic organisms are non-interactive there is extensive dialogue on whether this is due to past competition or no competition past or present. Holmes (1973) suggested that non-interaction in parasite communities is due to component species initially occupying similar niches, but selective pressure to minimize interspecific competition resulted in niche differentiation. On the other hand, Rohde (1979) and Price (1980, 1984) consider parasites as specialists in an environment of abundant resources where they evolved their attributes as species independently. When specialist parasite species are assembled by chance colonizations into communities their niches are unlikely to overlap, but if they do overlap resource abundance will prevent competition.

The first hypothesis (Holmes 1973) invokes the "ghost of competition past" (Connell 1980) and is difficult to falsify (Strong 1984). It would be supported if there was reason to suspect that past competition occurred (Strong 1984). Two possibilities are the presence of species flocks segregated within the intestine (Holmes 1973) and the abuttment of occupied niches along relevant resource gradients such as the intestine (Holmes and Price 1980). Species flocks were not observed in pike, and intestinal distributions of pike helminths ranged from having considerable overlap to having wide separations between infrapopulations of species pairs. Other support for the hypothesis of past competition would be evidence that potential competitors co-occurred frequently and in sufficient numbers

to result in selection pressure to minimize competition (Connell 1980). Helminths of pike frequently co-occurred, probably as a result of the utilization of a small number of intermediate host species as food by pike. However, intensities were generally low relative to resource availability and therefore the conditions that would produce interspecific competition were rare. Quite different patterns and rates of parasite recruitment than those observed in this study would be required to produce selective pressure. Intraspecific restrictions such as those found for T. crassus would also tend to reduce the intensity of interspecific contact.

The second hypothesis (Rohde 1979; Price 1980, 1984) would be falsified if resources were shown to be limiting in natural situations and if interspecific competition for those resources reduced a species' survival status. Previous studies did not address this since resources were not quantified. Based on the findings of this study it is suggested that three resources important for survival and reproduction of intestinal parasites were generally abundant in pike, although short-term limitations might occur. Furthermore, the interspecific interactions that were observed did not produce detectable reductions in the survival status of the participating species.

This study does not support the interpretation of past competition as a structuring mechanism for helminth communities in pike, unless one concludes that past competition first structured the parasite community when more species were present and then through a series of extinctions left empty or underutilized niches. The abundance of

resources and low intensities of parasites found in this study suggest that the species-specific attributes which provided structure to this primarily non-interactive community evolved independently under selection pressures other than interspecific competition. Whether this conclusion is generalizable to helminth communities in other host species is not known yet, since additional information is needed on resource availability from other natural parasite communities. Nevertheless, since seasonality, geographic variability, and overdispersion are common attributes of parasite transmission it is predicted that subsequent studies evaluating resource availability in conjunction with helminth community structure will reach similar conclusions.

CHAPTER 3: TRIAENOPHORUS CRASSUS IN SPAWNING NORTHERN PIKE

ABSTRACT

Infrapopulation sizes, growth, maturation, and fecundity of Triaenophorus crassus in spawning northern pike did not vary significantly over four consecutive years at Falcon L., Manitoba. Parasites were overdispersed in the sampled pike and the relationship was described by a negative binomial frequency distribution with k= 0.64. Type 1 individuals of T. crassus were defined as those not containing eggs. These were the most common worms and their dry mass was 0.06-73.9 mg. Type 2 individuals possessed eggs but did not release them when placed in water. These were least common and their dry mass was 1.20-72.8 mg. Type 3 individuals possessed eggs and shed them when placed in water. Their dry mass was 5.69-124 mg and they released 5,000- 4,400,000 eggs (mean= 489,000). Egg release by individual worms had a highly skewed distribution and half of all eggs released could be accounted for from five percent of all worms. Total release of eggs of T. crassus per infrapopulation was also skewed, due to overdispersion of parasite numbers among hosts, and less than nine percent of spawning pike could provide half of all parasite eggs released. The large number of type 1 worms indicated that many worms failed to reproduce before their death. A model was developed in which seasonal variation in predator-prey interactions affects timing of recruitment and the probability of a recruit successfully maturing in time to release eggs declines as the following spring approaches.

The model suggests that the presence of immature worms in spring can result from host feeding behavior, but also indicates that T. crassus may take longer than one year to mature in northern lakes.

INTRODUCTION

Cestodes utilizing aquatic poikilotherms as the definitive host frequently have a life cycle in which the period of egg release is brief relative to the length of prepatent development (Kennedy 1983). The Triaenophorus spp. cestodes represent one of the more extreme cases of this strategy, where egg release coincides with a brief period in the spring when their hosts, primarily Esox spp., are spawning in shallow waters. It was suggested (Miller 1952; Lawler 1969) that for T. crassus this behavior maximizes the likelihood that eggs would hatch in proximity to littoral populations of copepods, the first intermediate host, and that infected copepods in this area would subsequently be eaten by young coregonid fishes, the second intermediate host.

Seasonal changes in numbers and maturation of Triaenophorus spp., particularly T. nodulosus, have been extensively studied. Recruitment of plerocercoids of T. nodulosus by northern pike Esox lucius occurs primarily from March to October in Eurasia (Michajlow 1933; Chubb 1963; Kuperman 1973) and gonadal development begins in September or October (Michajlow 1933; Chubb 1963; Borgstrom 1970; Kuperman 1973). Worms with eggs in the uterus are present as early as December (Borgstrom 1970) and may remain until March or June depending upon locality but egg release occurs mainly during the latter part of that

interval (Chubb 1963; Scheuring, in Michajlow 1962; Borgstrom 1970; Kuperman 1973). The development of T. nodulosus in North America follows a similar sequence although most events occur about one month later (Miller 1943a). Triaenophorus crassus has been studied mainly in North America, and is recruited during May or June, with gonadal development beginning in November or December and eggs present in the uterus from December to June (Ekbaum 1937; Miller 1943a). Miller (1943a) described in vivo egg release by T. crassus. Triaenophorus spp. detach from the intestine during or shortly after egg release (Michajlow 1933; Miller 1943a; Chubb 1963; Borgstrom 1970; Kuperman 1973) indicating that there is an annual life cycle culminating in a brief period of egg release before death.

Since the entire lifetime fecundity of these parasites is expressed during the brief period of pike spawning, it is surprising that little quantitative information is available on the characteristics of these terminal parasite infrapopulations. Some information is available on age structure of worms during the last few weeks, as indicated by proportions of worms in various developmental states (Michajlow 1933; Chubb 1963; Borgstrom 1970; Kuperman 1973), indicating that immature worms (in various states of development but not possessing eggs), egg-bearing worms that do not release their eggs when placed in water, and egg-bearing worms that release their eggs when placed in water, may all be present in varying proportions. Miller (1943a) determined that T. crassus can release 1,750,000 eggs. However, much basic information is lacking on annual and inter-host variability of parasite numbers and characteristics, individual variability in growth and fecundity of Triaenophorus spp., or the

relationship between size and fecundity.

The purpose of this study was to evaluate: (1) annual stability in attributes of T. crassus infrapopulations in spawning northern pike, (2) inter-host variability in numbers, size, and fecundity of T. crassus, (3) individual variability in mass and fecundity of T. crassus, and (4) the relationship of these three to transmission of this parasite.

MATERIALS AND METHODS

DATA COLLECTION

Northern pike were collected, using nets stretched completely across an inlet creek to Falcon L., Manitoba (Fig. 12), during their spawning runs in the spring of 1981-1984. Each year nets were first set within two days of the first open water appearing at the mouth of the creek and 1-3 subsequent collections were made (Table 10). The same net (10 cm stretched mesh) was used in all collections. Pike were removed from the net within 1.5 h of setting, and killed by a blow to the head. The intestinal tract was removed and placed in a plastic bag on ice until examination. Host data collected were fork length, mass, sex, and reproductive state. The cleithrum was removed for ageing.

Examination of intestinal tracts and recovery of parasites was done within 2 h of host death. The intestine was pinned onto a waxed board and measurements taken between: (1) the pylorus and intestinal-rectal valve, and (2) the intestinal-rectal valve and anus. The intestine was slit longitudinally and care taken not to cut parasites. The intestine was then pinned open to expose worms in situ, and

Figure 12. Collections sites of spawning northern pike at Falcon Lake, Manitoba.

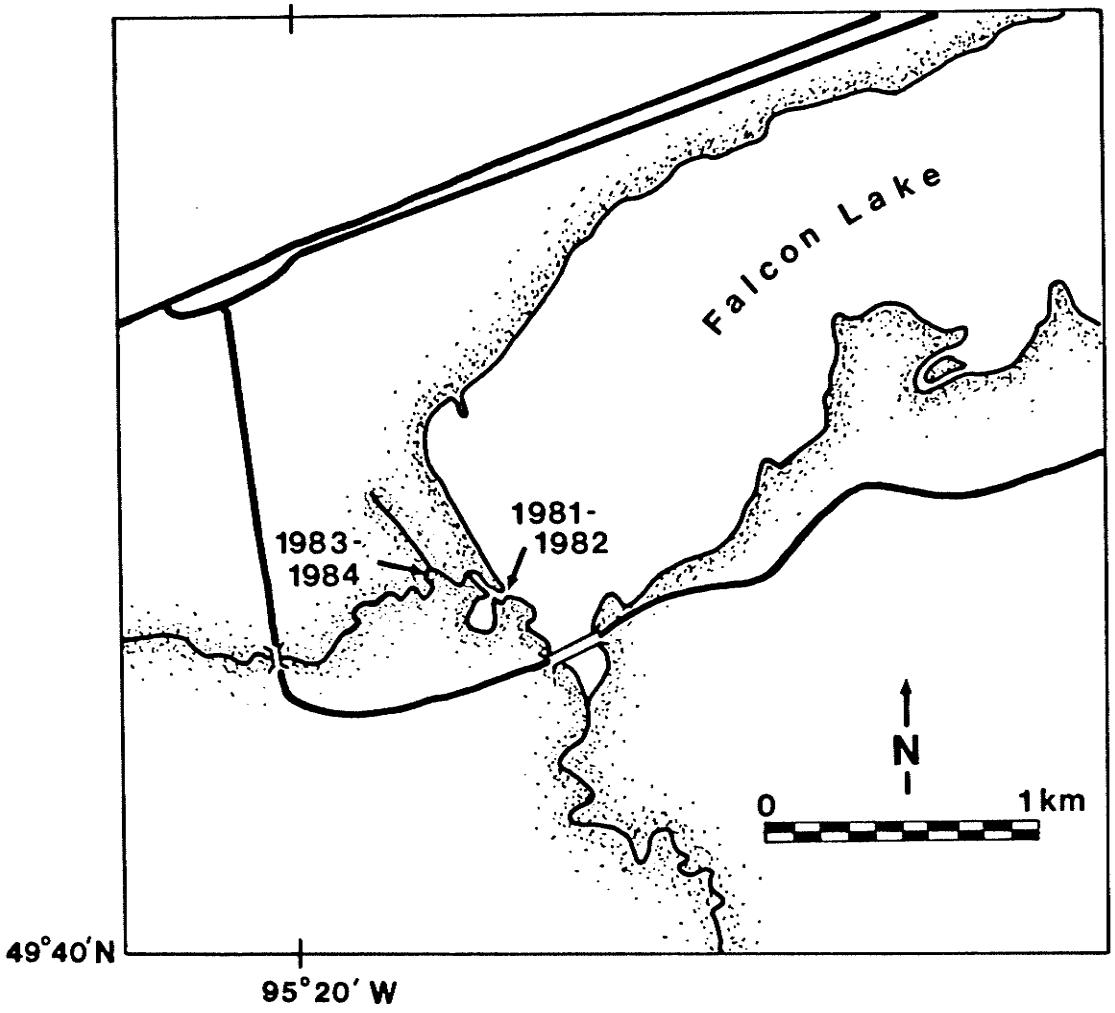

Falcon Lake
1983-
1984
1981-
1982
N
0
1 km
49°40'N
95°20' W

Table 10. Northern pike collections at Falcon L.

Year	Date	Water temp.[a]	Hours net set	No. pike / h
1981	5 April	7	1	8
	9 April	-	5	6
	30 April	-	1	0
1982	20 April	-	2	7.5
	27 April	-	2.5	4.4
1983	19 April	2	1	0
	22 April	6	4	4.5
	25 April	8	1	8
	29 April	3	1.5	0
1984	15 April	-	3	2.3
	17 April	-	2	4.5
	20 April	12	2	3.5

[a] Degrees C at 0.3 m depth.

irrigated with dechlorinated water to facilitate individual removal of worms (dechlorinated water was used in all subsequent handling of worms). The distance from the pylorus to the scolex of each T. crassus was measured and the entire worm was removed and placed in a numbered 15x125 mm test tube containing 10 ml water. Through this individual numbering a set of data unique to each worm was assembled in the remainder of the study. After removal of all T. crassus the approximate numbers and locations of other helminths were recorded and the intestine rebagged and frozen for later examination. Test tubes containing T. crassus were placed on ice for transport back to the laboratory. Each worm from the 1981-1984 collections was placed in a numbered 100 mm plastic petri dish, and all eggs in a test tube were transferred to the same dish. The dishes were placed in a refrigerator at 8C overnight to permit completion of egg release. This temperature was chosen because it was within the range that the worms experienced in spawning pike (Table 10).

The following morning, ca. 20 h after removal from the pike, the worms, now dead, were removed from the petri dishes and floated in a pan of room temperature (ca. 20C) water to measure relaxed length (lengths were not measured in 1981). The scolex of each worm was examined to confirm species identification. The presence or absence of eggs remaining in the uterus was noted but counts were not made (worms which released eggs generally retained less than ca. 5%). Each worm was then placed on a numbered glass slide, dried at 70C for 48 h, and its mass determined to the nearest 0.01 mg. The slide was weighed with the dried worm and reweighed immediately after scraping off the worm. This minimized possible errors in taring the balance, or

changes in slide weight due to chipping or moisture uptake that might occur if slides had been preweighed.

Several techniques were tried in 1981 to assess fecundity, but to avoid biasing comparisons with samples from subsequent years this data was not used. Fecundity was assessed in 1982-1984 as the total number of eggs released over a 20 h period, first into a test tube on ice (ca. 5C) for ca. 6 h and then in a petri dish held at 8C in the dark for the remainder. Preliminary observations found that worms usually released most of their eggs within the first few hours after being placed in water; relatively few additional eggs were released overnight. The degenerative changes noted by Miller (1943a) in T. crassus, and assumed to be worms that had previously shed eggs, were not found in any worms recovered from the pike in my study. Consequently, I assumed that eggs recovered represented lifetime fecundity of the worms.

Eggs were also required for controlled studies on subsequent life cycle stages (chapters 4, 5, and 6) and therefore had to be counted quickly and accurately. Egg counts were made as follows. The contents of a petri dish were placed in a beaker, the water volume brought up to 100 ml, and stirred with a magnetic stir bar for 30 s. Serial dilutions ranging from 0.5-10.0% were made, depending on egg numbers, so that 50-500 eggs were in a final 1.0 ml sample. The 1.0 ml sample was placed on a gridded petri dish to count eggs. Total dilutions ranged from 1/100 to 1/20,000. Accuracy of the procedure was tested at these two extremes. At a 1/100 total dilution, 10 replicate estimates from a common initial source of eggs over a

magnetic stirrer gave counts of 356 ± 17 eggs, indicating that ca. 95% of counts (± 2 SD) were within 10% of the mean. At a 1/20,000 total dilution, 10 separate replications of the entire dilution procedure gave a count of 69 ± 10 eggs, indicating that ca. 95% of counts (± 2 SD) were within 30% of the mean.

DATA ANALYSIS

The distribution of T. crassus among pike was tested for fit to the Poisson and negative binomial frequency distributions, using the method of maximum likelihood, with programs in the APL public library of the University of Manitoba Computer Services. A X^2 test was used for fit to the Poisson, whereas fit to the negative binomial was tested with the U statistic (Poole 1974). Length-mass relationships were evaluated using analysis of covariance (ANCOVA) programs in the APL public library.

Variation in worm mass and fecundity was analyzed using the NESTED analysis af variance (ANOVA) procedure in SAS, in which levels of the hierarchy were: (1) worms attached within an intestinal section (5% section of intestinal length corresponding to those used in chapters 1 and 2 were calculated from the raw measurements taken during worm collection), (2) section within a fish, (3) fish within years, and (4) year of collection. A model with random effects at all levels was used, and significance of added variance at each level in the nested ANOVA was tested using procedures in Sokal and Rohlf (1981). Trends

of changing proportions of mature to immature T. crassus relative to intestinal section were examined using a X^2 test for linear regression in an Rx2 table (Steele and Torrie 1980) in which weights were assigned equivalent to the distance in sections from one of three reference points used.

RESULTS

The dates on which pike were collected in the shallows varied yearly within a 2 wk period (Table 10). All pike had fully-developed gonads; most released eggs or sperm during handling.

A total of 1284 T. crassus was collected; 398 in 1981, 272 in 1982, 295 in 1983, and 319 in 1984. These were classified into one of three categories. Type 1 were thin worms with or without developed gonads but without eggs if gonads had formed. Occasionally, low numbers of eggs (< 5000) were found in a dish with a type 1 worm, indicating contamination by eggs released from other worms in the host fish. Type 2 worms were thicker and contained eggs in the uterus, but few or no eggs were released into water overnight. Since type 1 worms had up to 5000 contaminating eggs, type 2 worms included those in which up to 5000 eggs were present, but they were treated in all analyses as having released no eggs. Type 3 worms were the thickest of the three, contained eggs in the uterus, and released most or all of their eggs when placed in water (at least 5000 eggs). The scoleces of almost all (> 99%) of the three types of worms were firmly embedded in the intestinal wall.

Since the numbers of T. crassus per pike did not vary significantly among the four years of the study (Table 11) all worms and hosts were pooled to examine the distribution of worms among

Table 11. Numbers of T. crassus per northern pike at Falcon L.

1981	1982	1983	1984
2.236 ± 1.398[a] (N= 26)	2.191 ± 1.756 (N= 17)	1.811 ± 1.631 (N= 26)	2.248 ± 1.866 (N= 19)

ANOVA[b]

Source	df	SS	MS	Significance test
Among years	3	3.2028	1.0676	F= 0.653; P= 0.597
Within years	84	137.3982	1.6357	
Total	87	140.6010		

[a] $\bar{X}$ ± SD of ln(no. worms + 1); (N= no. pike).

[b] Data were transformed by ln(no. worms + 1).

hosts. Total worm counts (Fig. 13) or counts of worms segregated by type (Fig. 14) did not follow a Poisson distribution ($P < 0.001$). The negative binomial distribution provided an acceptable fit for total numbers (Fig. 13) ($U = -68.6$; $var(U) = 124$). A good fit to the negative binomial was also observed for type 1 worms (Fig. 14A) ($U = -36.0$; $var(U) = 67.4$) and type 3 worms (Fig. 14C) ($U = -20.8$; $var(U) = 70.8$) but not type 2 worms (Fig. 14B) ($U = -1.08$; $var(U) = 0.35$).

The three types of T. crassus occurred in similar proportions during the three years of collection in which they were distinguished ($X^2 = 9.34$; $df = 4$; $P = 0.053$). Type 1 worms were most common while type 2 were least common (Table 12). Type 3 worms comprised only 22-31% of all T. crassus, but they contributed on average 62% of all mass.

A summary of measurements of the three types of T. crassus is presented in Table 13 and shows that not only did all measurements have an extensive range but there was also considerable overlap in ranges between the types. The difference in mass between types was highly significant (ANOVA using ln(mass): $F = 515$; $df = 2, 853$; $P < 0.001$) and differences between types accounted for 55% of total variation. Several additional analyses were performed to examine mass of individual T. crassus within each type.

The growth form of T. crassus was examined using length-mass relationships. The use of $\sqrt{mg}$ vs. mm produced a linear relationship in which the residuals by visual examination were independently and normally distributed about the regression line. Type 1 worms (Table 14) seemed to exhibit a similar length-mass relationship among years but this could not be tested statistically due to heterogeneity about

Figure 13. Frequency distribution of T. crassus among spawning northern pike at Falcon Lake, Manitoba. Dots indicate expected frequencies from negative binomial distribution. Maximum likelihood estimate of k is shown.

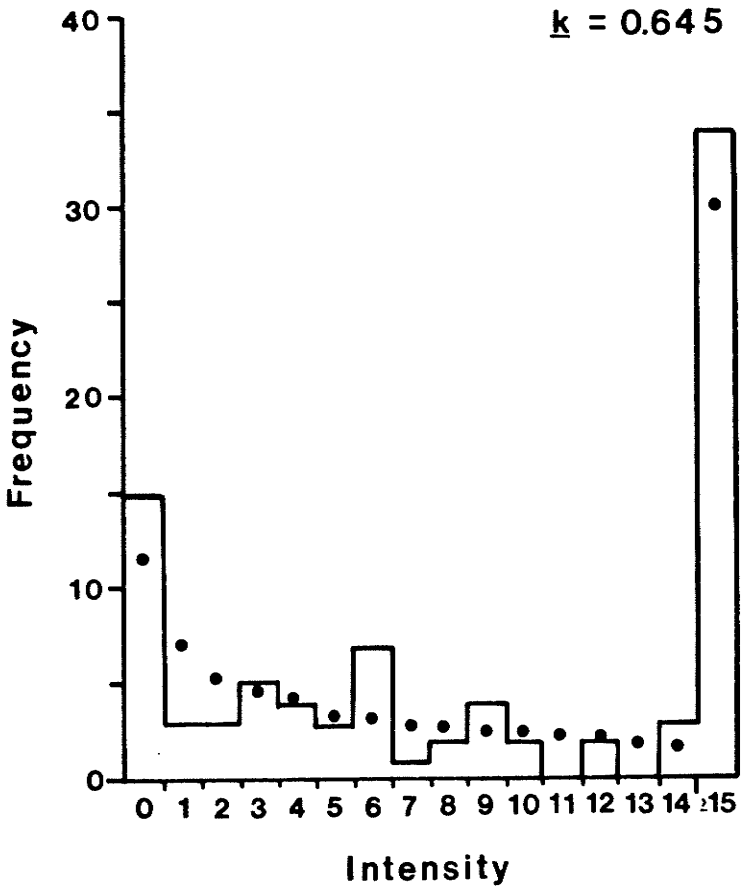

k = 0.645
40
30
20
10
0
Frequency
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Intensity

Figure 14. Frequency distributions of T. crassus, segregated by type, among spawning northern pike at Falcon Lake, Manitoba. A. Type 1. B. Type 2. C. Type 3. Dots indicate expected frequencies from negative binomial distribution. Maximum likelihood estimates of k are shown.

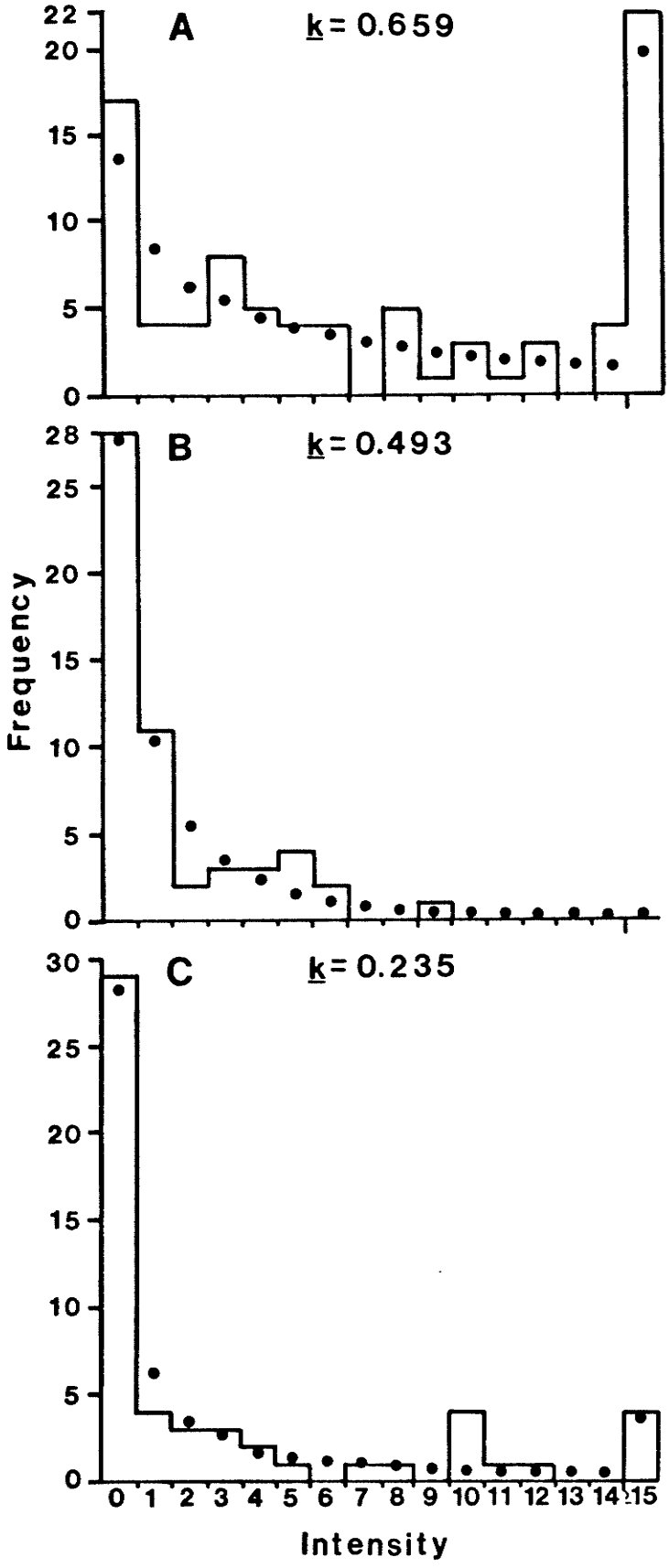

A
k = 0.659
B
k = 0.493
C
k = 0.235
Frequency
Intensity
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Table 12. Frequencies of three types of T. crassus in northern pike over three years at Falcon L.

Type	1982	1983	1984
1	129 (59)[a]	200 (70)	221 (69)
2	21 (10)	29 (10)	27 (8)
3	64 (31)	58 (20)	70 (22)
Total	214	287	318

[a] N (%)

Table 13. Traits of adult T. crassus, pooled across hosts and years.

	Type 1	Type 2	Type 3
Length (mm)	168 ± 107[a]	283 ± 120	374 ± 135
	(532)	(77)	(190)
	[5-600]	[65-700]	[115-870]
Mass (mg)	5.53 ± 6.30	19.2 ± 15.1	37.9 ± 22.4
	(818)	(75)	(188)
	[0.06-73.9]	[1.20-72.8]	[5.69-124]
Fecundity	-	-	489000 ± 593000
(eggs/worm)	-	-	(192)
	-	-	[5000-4,400,000]

[a] $\bar{X}$ ± SD, (N), [minimum-maximum]

Table 14. Length-mass relationships of type 1 T. crassus from northern pike over three consecutive years. Length (X) is mm and mass (Y) was transformed by $\sqrt{mg}$.

Year	Independent regression results			
	Y- intercept	Slope (b)	SE(b)	N
1982	0.7513	0.006645	0.0002907	106
1983	0.6936	0.007436	0.0002874	197
1984	0.6056	0.008546	0.0003639	212

the independent regression lines (Bartlett's X^2= 22.0; df= 2; P< 0.001). The slope of the length-mass relationship for type 2 worms did not differ significantly among years but there were significant annual differences in elevation of the regression line (Table 15): in 1984 type 2 worms were heavier at a given length than in the previous two years. Type 3 T. crassus, similar to type 2, did not have significant annual variability in the slope of the length-mass relationship but the elevations differed (Table 16): in 1982 type 3 worms were lighter at a given length than in the following two years. Data were pooled across years to compare type 2 and type 3 T. crassus (Table 17). Type 3 worms had similar slopes but were heavier at a given length than type 2 worms (Table 17).

A preliminary examination of pooled data indicated a possible relationship between section of worm attachment, and worm mass and fecundity (Fig. 15), particularly for type 3 worms which were significantly smaller and less fecund when attached more posteriad. To test whether this relationship might be due to bias in the source of worms used to form the regression (either in the fish they came from or in the year in which they were collected) nested ANOVAs were used to identify sources of added variation in mass of the three types of T. crassus (Tables 18, 19, and 20A). Small but significant added variance components in mass of type 1 worms could be attributed to section of intestinal attachment and host fish, but not to year of collection (Table 18). The mass-frequency distribution of type 1 worms was highly skewed (Fig. 16). The mass of most worms corresponded to mass of plerocercoids within cisco collected from Southern Indian L.; only 18% were larger than the largest plerocercoid

Table 15. Length-mass relationships of type 2 T. crassus from northern pike over three consecutive years. Length (X) is mm and mass (Y) was transformed by $\sqrt{mg}$.

Independent regression results

Year	Y- intercept	Slope (b)	SE(b)	N
1982	0.7538	0.01027	0.001966	19
1983	0.8637	0.01084	0.001869	29
1984	2.9776	0.006038	0.001883	27

ANCOVA

Test of slopes[a]

Source	df	SS	MS	Significance test
Among years	2	4.522	2.2609	F= 1.942; P= 0.149
Within years	69	80.347	1.1644	

Test of intercepts[b]

Source	df	SS	MS	Significance test
Among years	2	12.904	6.4522	F= 5.398; P= 0.007
Within years	71	84.869	1.1953	

[a] Homogeneity of residuals about independent regressions: X^2= 1.51; df=2; P= 0.470.

[b] Homogeneity of residuals assuming common slope (b=0.008940): X^2= 2.25; df= 2; P= 0.324.

Table 16. Length-mass relationships of type 3 T. crassus from northern pike over three consecutive years. Length (X) is mm and mass (Y) was transformed by $\sqrt{mg}$.

Independent regression results

Year	Y- intercept	Slope (b)	SE(b)	N
1982	1.965	0.01002	0.001237	60
1983	2.537	0.008532	0.001300	56
1984	2.713	0.009338	0.001090	70

ANCOVA

Test of slopes[a]

Source	df	SS	MS	Significance test
Among years	2	1.0646	0.5323	F= 0.358; P= 0.705
Within years	180	267.8025	1.4871	

Test of intercepts[b]

Source	df	SS	MS	Significance test
Among years	2	11.346	5.673	F= 3.840; P= 0.023
Within years	182	268.867	1.477	

[a] Homogeneity of residuals about independent regressions: X^2= 1.97; df= 2; P= 0.373.

[b] Homogeneity of residuals assuming common slope (b= 0.009241): X^2= 2.20; df= 2; P= 0.333.

Table 17. Length-mass relationships of type 2 and type 3 T. crassus from northern pike, pooled over three consecutive years. Length (X) is mm and mass (Y) was transformed by $\sqrt{mg}$.

Type	Y- intercept	Slope (b)	SE(b)	N
Type 2	1.5111	0.009172	0.001145	75
Type 3	2.2912	0.009610	0.0006719	186

ANCOVA

Test of slopes[a]

Source	df	SS	MS	Significance test
Among types	1	0.1502	0.1502	F= 0.1021; P= 0.745
Within types	257	377.9865	1.4708	

Test of intercepts[b]

Source	df	SS	MS	Significance test
Among years	1	40.218	40.218	F= 27.44; P< 0.001
Within types	258	378.137	1.466	

[a] Homogeneity of residuals about independent regressions: X^2= 0.42; df= 1; P= 0.517.

[b] Homogeneity of residuals assuming common slope (b= 0.009508): X^2= 0.38; df= 1; P= 0.538.

Figure 15. Mass (M) and fecundity (F) of T. crassus relative to section of attachment of the worm in the intestine of spawning northern pike at Falcon Lake, Manitoba. Points represent $\bar{X} \pm$ SD.

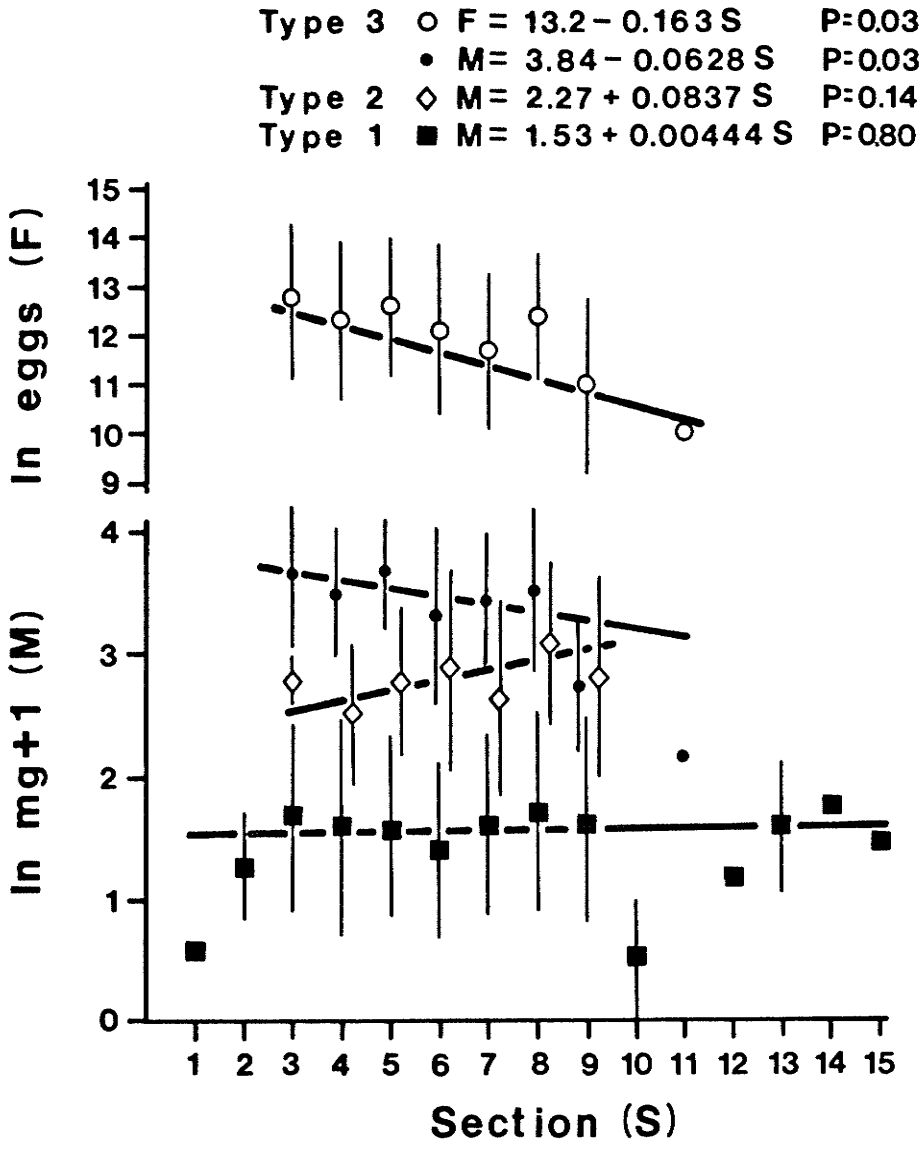

Type 3 ○ F = 13.2 − 0.163 S P=0.03
• M= 3.84 − 0.0628 S P=0.03
Type 2 ◇ M= 2.27 + 0.0837 S P=0.14
Type 1 ■ M= 1.53 + 0.00444 S P=0.80
ln eggs (F)
ln mg+1 (M)
15
14
13
12
11
10
9
4
3
2
1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Section (S)

Table 18. Nested analysis of variance on dry mass of type 1 T. crassus. Data were transformed by ln(mg+1). All worms included.

Source	df	MS	% Variance component	Significance test[a]
Year	3	1.97485	0.00	F'=0.761; df=3, 46; P= 0.525
Fish	64	1.63638	12.30	F'=2.556; df= 64, 44; P= 0.001
Section	112	0.64024	7.90	F= 1.326; df= 112, 638; P= 0.020
Error	638	0.48282	79.80	
Total	817	0.60024	100.00	

[a] F' is approximate test using Satterthwaite approximation.

Table 19. Nested analysis of variance on dry mass of type 2 T. crassus. Data were transformed by ln(mg+1). All worms included.

Source	df	MS	% Variance component	Significance test[a]
Year	2	2.52697	6.10	F'= 1.530; df= 2, 19; P= 0.241
Fish	22	1.11466	61.17	F'= 9.770; df= 22, 8; P= 0.002
Section	16	0.12670	0.00	F= 0.660; df= 16, 34; P= 0.811
Error	34	0.19080	32.73	
Total	74	0.51474	100.00	

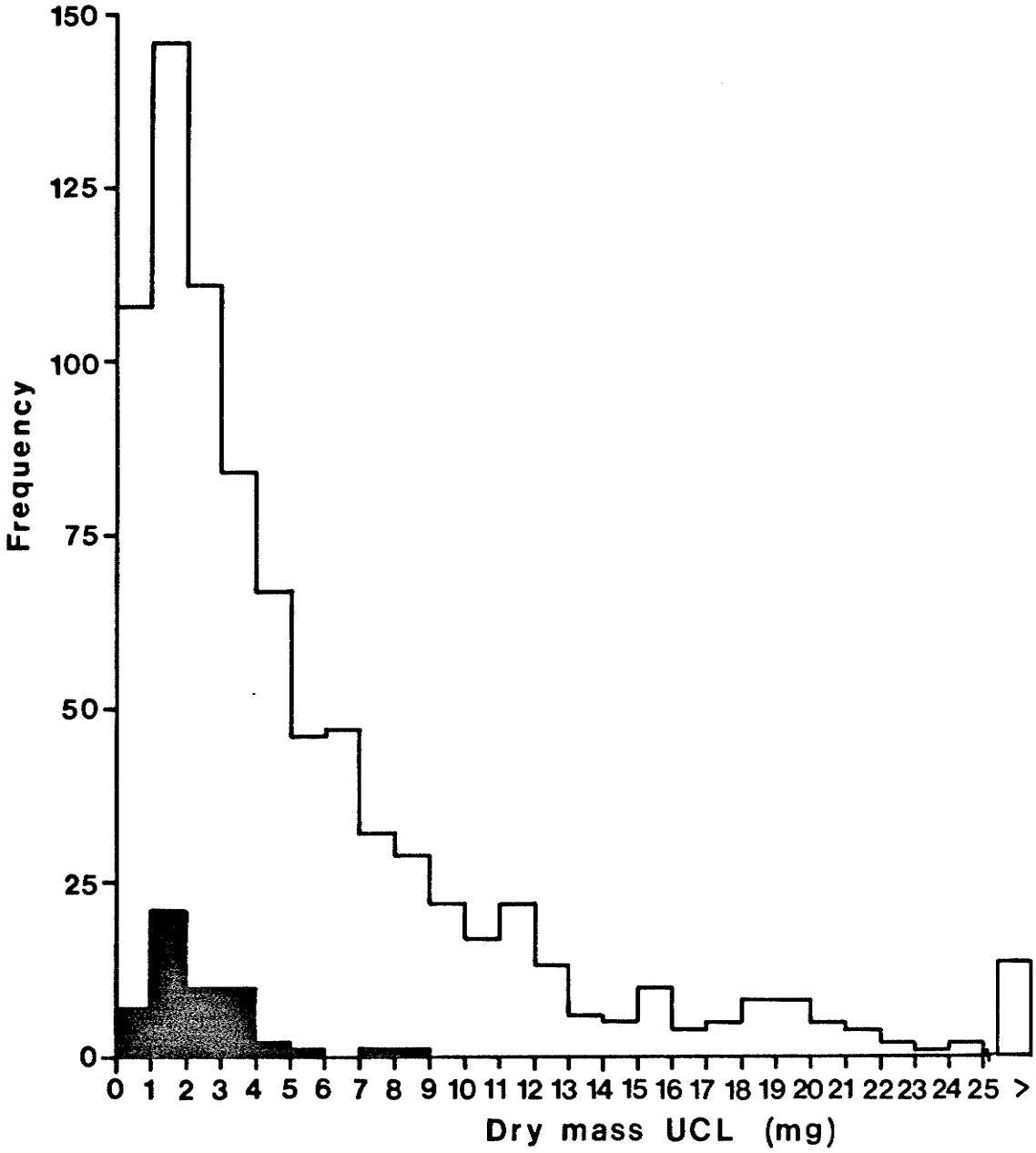
[a] F' is approximate test using Satterthwaite approximation.

Table 20. Nested analysis of variance on dry mass of type 3 T. crassus. Data were transformed by ln(mg+1). A. All worms included. B. Only worms from lineages used in chapter 7.

Source	df	MS	% Variance component	Significance test[a]
A.				
Year	2	3.83407	11.48	F'= 3.780; df= 2, 12; P= 0.053
Fish	22	0.65048	7.92	F'= 1.469; df= 22, 25; P= 0.176
Section	38	0.39721	13.46	F= 1.504; df= 38, 125; P= 0.049
Error	125	0.26415	67.14	
Total	187	0.37482	100.00	
B.				
Year	1	0.07866	0.00	F'= 0.272; df= 1, 5; P= 0.627
Fish	11	0.25045	6.25	F'= 1.159; df= 11, 8; P= 0.427
Section	10	0.21181	14.57	F= 1.282; df= 10, 15: P= 0.321
Error	15	0.16520	79.18	
Total	37	0.20080	100.00	

[a] F' is approximate test using Satterthwaite approximation.

Figure 16. Frequency distribution of mass of type 1 T. crassus at Falcon Lake, Manitoba. Solid bars, mass of plerocercoids of T. crassus from cisco at Southern Indian Lake, Manitoba. UCL, upper class limit.


150
125
100
75
50
25
0
Frequency
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 >
Dry mass UCL (mg)

(Fig. 16). Host fish provided a large added variance component for mass of type 2 worms (Table 19). All three sources provided small but significant or marginally-significant added variance components to mass of type 3 worms (Table 20A). The mass of type 3 T. crassus varied considerably, from 6-124 mg (Fig. 17A) but most were in the 10-50 mg range.

The fecundity of type 3 T. crassus did not vary among sections of attachment, host fish, or year of collection (Table 21A) and averaged ca. 500,000 eggs with a median of ca. 300,000 eggs, although the distribution of fecundities was markedly skewed (Fig. 17B) and up to 4,400,000 eggs were released. The average fecunditiy of types 1-3 combined was 120,000 eggs/worm. Mass-specific fecundity of type 3 T. crassus ranged from 260-78,500 eggs/mg. Fecundity was positively correlated with worm mass (r^2= 0.43; df= 230; P< 0.001) although about half of the variability in fecundity was not explained by mass. Half of all fecundity was from < 5 % of all worms sampled (Fig. 18A). The total fecundity per infrapopulation of T. crassus did not vary significantly among years (Table 22). Maximum fecundity was 15,000,000 eggs/host, and average fecundities were 4,000,000 eggs/host that harbored at least one type 3 worm, 2,000,000 eggs/pike infected with T. crassus, and 1,700,000 eggs/spawning pike. Overdispersion in fecundity per infrapopulation was also noted (Fig. 18B). Less than 9% of all sampled pike provided half the total T. crassus fecundity.

The relationship between section of attachment and maturation was tested by relating distance (in sections) from the section of attachment to three reference points: (1) the pylorus, (2) section 5

Figure 17. Frequency distributions of mass and fecundity of type 3 T. crassus at Falcon Lake, Manitoba. A. Mass. B. Fecundity. Solid bars, distributions of lineages used in analyses of chapter 7. UCL, upper class limit.

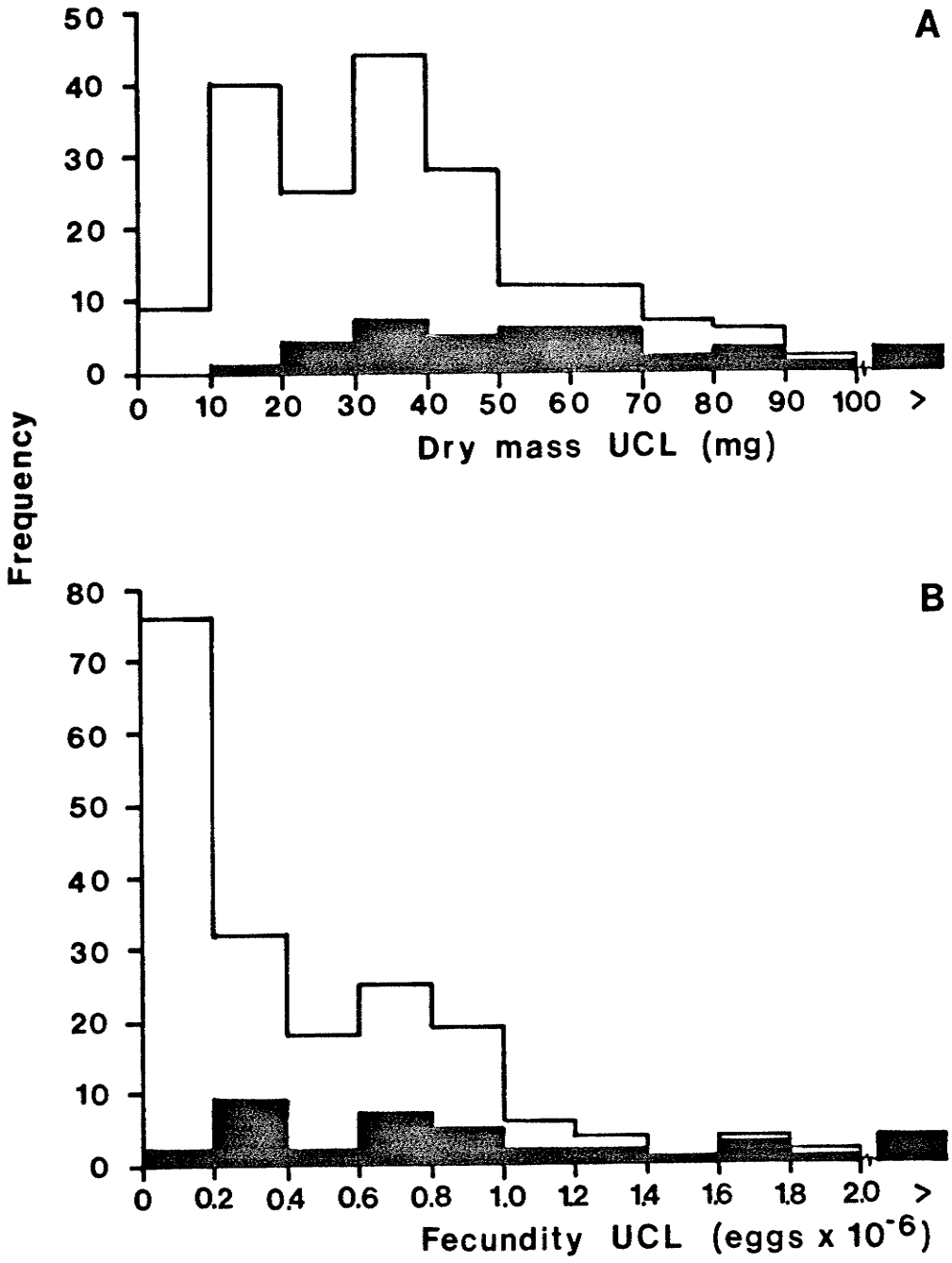

A
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100 >
Dry mass UCL (mg)
Frequency
B
80
70
60
50
40
30
20
10
0
0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 >
Fecundity UCL (eggs x 10-6)

Table 21. Nested analysis of variance on fecundity of type 3 T. crassus. Data were transformed by ln(eggs+1). A. All worms included. B. Only worms from lineages used in chapter 7.

Source	df	MS	% Variance component	Significance test[a]
A.				
Year	2	2.50651	0.00	F'= 0.532; df= 2, 8; P= 0.611
Fish	22	5.96647	12.53	F'= 1.780; df= 22, 24; P= 0.085
Section	38	5.00385	9.24	F= 1.403; df= 38, 129; P= 0.141
Error	129	2.60552	78.23	
Total	191	1.99780	100.00	
B.				
Year	1	0.38520	0.00	F'= 0.343; df= 1, 5; P= 0.587
Fish	11	0.96747	0.00	F'= 0.996; df= 11, 9; P= 0.511
Section	10	0.92906	39.04	F= 1.982; df= 10, 15; P= 0.111
Error	15	0.46877	60.96	
Total	37	0.73918	100.00	

[a] F' is approximate test using Satterthwaite approximation.

Figure 18. Frequency distributions of fecundity of T. crassus individuals and infrapopulations. A. Fecundity of individual worms. B. Total fecundity of T. crassus within the infrapopulation in a host. Type 1 and type 2 T. crassus did not release eggs.

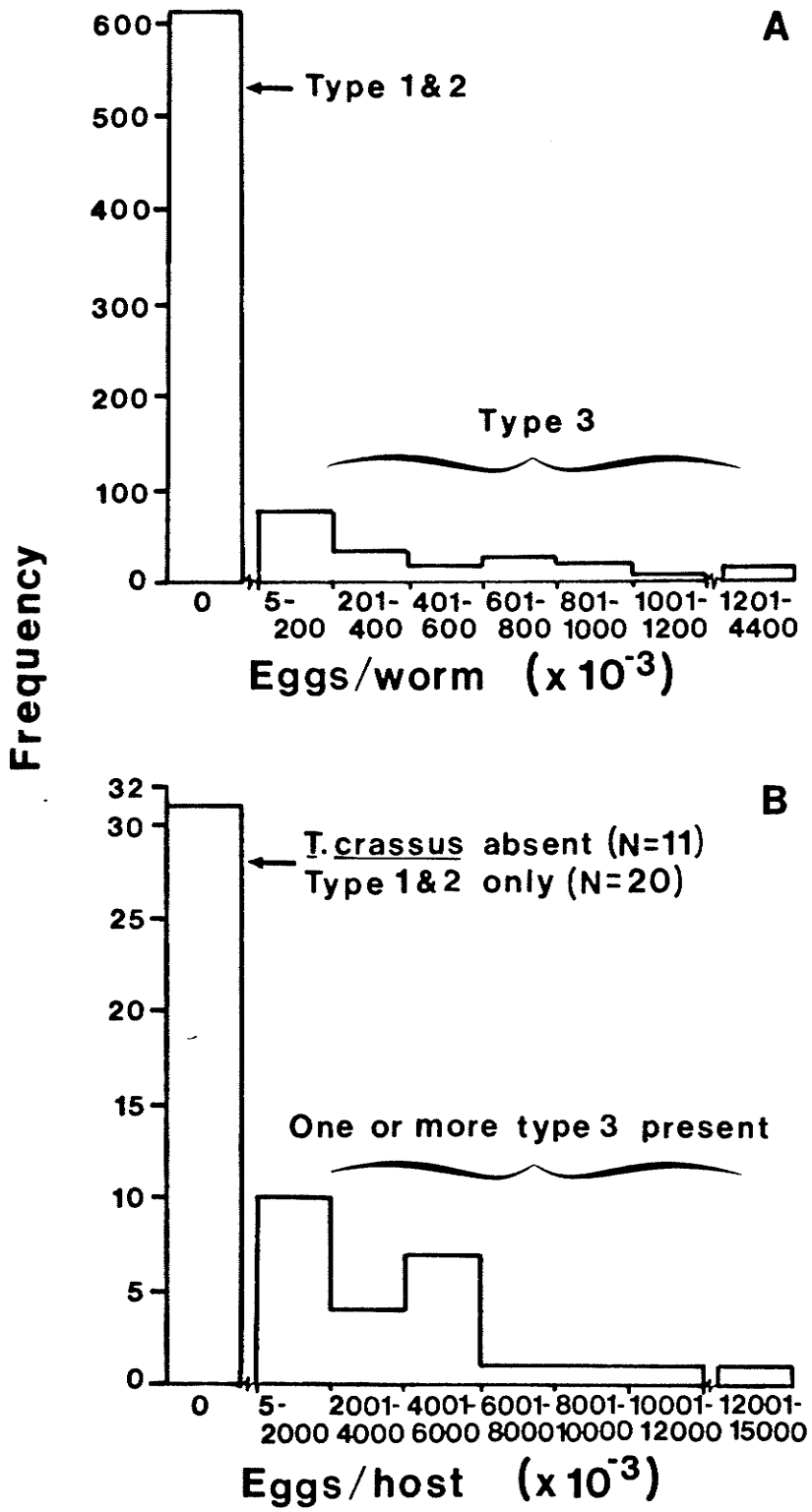

A
600
500
400
300
200
100
0
Type 1&2
Type 3
0
5-200
201-400
401-600
601-800
801-1000
1001-1200
1201-4400
Eggs/worm (x 10-3)
Frequency
B
32
30
25
20
15
10
5
0
T. crassus absent (N=11)
Type 1&2 only (N=20)
One or more type 3 present
0
5-2000
2001-4000
4001-6000
6001-8000
8001-10000
10001-12000
12001-15000
Eggs/host (x 10-3)

Table 22. Total number of eggs released by all T. crassus within a host northern pike. Data are only from pike with at least one egg-releasing T. crassus.

1982	1983	1984
15.1 ± 1.19[a] (N= 5)	14.1 ± 1.41 (N= 11)	14.2 ± 2.39 (N= 9)

ANOVA[b]

Source	df	SS	MS	Significance test
Among years	2	3.5220	1.7610	F= 0.545; P= 0.592
Within years	22	71.0754	3.2307	
Total	24	74.5968		

[a] $\bar{X}$ ± SD of ln(eggs + 1); (N= no. pike).

[b] Data were transformed by ln(eggs + 1).

(25% of intestinal length posteriad to the pylorus), and (3) the median attachment site within the individual host. Two measures of maturation were used: (1) the proportion of fecund (type 2 and 3) to non-fecund (type 1) worms, and (2) the proportion of egg-releasing (type 3) to non-releasing (type 2) fecund worms. There was a significant linear relationship between the proportions of fecund vs. non-fecund worms when distance was measured relative to the pylorus (Table 23). In sections 1-4, 43% of 231 worms were fecund, in sections 5-8, 30% of 923 were fecund, and in sections 9-15, 19% of 42 were fecund. Worms attached various distances from their own infrapopulation medians exhibited significant differences in the proportion of fecund to non-fecund individuals, but these proportions did not vary linearly with distance from the median (Table 23). Proportions of egg-releasing to non-releasing fecund worms did not vary significantly with intestinal location.

Table 23. Maturation of T. crassus relative to attachment site in the intestine of northern pike.

Basis for site categorization[a]	Total X^2	X^2 due to linear regression
I A	32.7; df= 8; P< 0.001	5.12; df= 1; P= 0.024[b]
I B	3.97; df= 6; P= 0.681	
II A	8.97; df= 5; P= 0.110	
II B	2.49; df= 4; P= 0.646	
III A	14.6; df= 5; P= 0.012	0.556; df= 1; P= 0.456
III B	3.76; df= 4; P= 0.440	

[a] I, absolute section of attachment; II, absolute distance in sections from intestinal section 5; III, absolute distance in sections from median section of infrapopulation; A, type 1 relative to type 2+3; B, type 2 relative to type 3.

[b] Significantly higher proportion of type 2+3 in more anteriad sections.

DISCUSSION

There are several largely unresolved questions in parasitology regarding the causes and consequences of variability between individual parasites and between parasite infrapopulations, particularly for naturally-infected hosts. These include intrinsic and extrinsic sources of variability within parasite infrapopulations, the reasons for overdispersion of parasite numbers and characteristics, and the proportion of a parasite population that contributes to its biotic potential. Since this study was the first to include individual fecundities with a variety of other individual and population characteristics of a parasite in the definitive host I am able to discriminate between some of the causes of variability and speculate on their importance in parasite transmission.

INDIVIDUAL VARIABILITY

A wide range of sizes and fecundities was found in T. crassus from spawning pike. Considerable variation in lengths of T. nodulosus at spawning time are known (Michajlow 1933; Chubb 1963; Kuperman 1973) but length variations within developmental stages were not quantified in these studies. It is interesting that although over half of all

variability in mass of T. crassus was explained by worm type, the mass of individuals varied over 1- 2 orders of magnitude within each of the three types. There was extensive overlap of ranges between types, but on average types 1, 2, and 3 were of increasing mass. This supports earlier suggestions (Roberts 1961; Kumazawa and Suzuki 1983) that maturation of cestodes is largely independent of their size.

Lifetime parasite fecundity is difficult to assess on an individual basis for species with long periods of patency. Individual fecundities cannot be determined if more than one worm is present, and daily, seasonal, or individual variations in production of offspring (Braten 1966; Platt and Samuel 1978; Belosevic and Dick 1979; Kwong and Dobson 1982) make it difficult to accurately determine individual or lifetime fecundities. Estimates from several cyclophyllidean or pseudophyllidean cestodes suggest that egg production of tens or hundreds of thousands per worm per day may continue for months or years (Braten 1966; Kennedy 1983). The synchronized life cycle of T. crassus, and release of almost all eggs when placed in water, made possible the study of individual variability in lifetime fecundity in a natural parasite population. A maximum of 4,400,000 eggs/ T. crassus agrees with 1,750,000 eggs/worm reported by Miller (1943b), but more importantly mean fecundity was only 500,000 eggs; a lifetime fecundity comparable to the daily fecundity of many other cestodes (Braten 1966; Kennedy 1983). Individual fecundity of T. crassus was more variable (ranged over three orders of magnitude) than size (two orders), but knowledge of the extent of variability did not clarify its cause or function. Additional analyses were performed to suggest answers to these questions.

The construction of frequency histograms demonstrated large inequalities of size and fecundity among individuals in the population. A small proportion of worms accounted for a large proportion of total recruitment. Fecundity as measured in this study showed variable reproductive effort among individuals, under controlled conditions that were similar to conditions for T. crassus during natural transmission from pike to water (Miller 1943a), and thus provided a measure of fitness. This fitness (= fecundity) distribution was markedly skewed, and Dobson (1986) notes that natural selection is sensitive to the shape of fitness distributions and not their mean values. The true fitness distribution may be even more skewed. Type 2 worms were more slender and presumably contained fewer eggs than type 3 worms; if they were able to complete maturation and release their eggs on the spawning grounds they would add disproportionately to the low-fecundity classes. This markedly skewed fecundity distribution is of interest in the modeling of helminth population dynamics. Fecundity per individual is usually incorporated as a constant (Keymer 1982b) or as a declining function of mean intensity (Anderson 1982), yet in chapter 2 it was shown that fecundity correlated poorly with intensity.

Individual variability has both genetic and environmental causes. It was not possible to quantify genetically-based variability in this study but through the use of nested ANOVAs several environmental sources of variability could be evaluated. The environmental variables were year of collection, host fish occupied, and section of attachment within the intestine of the host fish. The first variable, year of collection, presumably incorporated abiotic and biotic

factors, while the remaining variables primarily dealt with the biotic environment of the parasite since it was assumed that within a lake in a given year pike and their parasites would experience a similar set of abiotic conditions. The exact mechanisms by which each of these levels might affect the parasite were not determined, and therefore these levels represented a series of black boxes. However, they were useful because they permitted assessment of three key components of the environment: (1) year-to-year variability, which is useful for considering long term stability in features of parasite transmission, (2) variation between hosts, which for parasites is equivalent to evaluating variation between isolated local populations, and (3) variations within hosts, which allows detection of heterogeneity within a local population. Identification of any of these black boxes as a source of variability in parasite characteristics would serve to focus future research efforts to determine the underlying mechanism. Variance components estimated the proportion of total variance in a characteristic accounted for by successive levels in this environmental hierarchy. Since confidence intervals for the variance components could not be calculated because unequal sample sizes were involved (Sokal and Rohlf 1981) they were interpreted as approximate values. These variables accounted for small and occasionally statistically significant variance. More interesting were the large error variance components, often up to 80%, which reflected variability among worms attached within a section (the lowest level of discrimination possible in this study). Even if the true error variance component was half of 80% it still leaves a substantial amount of unresolved variability. Some of this variance may relate to

age of worms but the best estimate of age that could be made is the assumption that type 1 worms were youngest and type 3 oldest, and that larger worms within a type were older than smaller worms. However, observations on the pattern of attachment by T. crassus described in chapter 2, and differences in worm maturation along the length of the intestine described here, suggest that worm age varied more between sections than within. Large infrapopulations of T. crassus were attached over a larger proportion of the intestine than small infrapopulations (chapter 2), indicating that new recruits established on the periphery. The alternate explanation that new recruits displaced established worms was not likely as the deep penetration of the scolex documented in chapter 1 suggested that T. crassus did not move freely in the intestine. It was observed that the proportion of immature worms increased significantly (51% to 81%) between anterior and more posterior attachment sites. Worms within a section may have been acquired at a similar time and matured at a similar rate. If, as this interpretation of the data suggests, age variations among worms in the same section are minimal, then the large error terms become increasingly difficult to attribute to environmental causes. Further evidence for the presence of large non-environmental sources of variability came from analysis of fecundity relative to worm mass; these two characteristics are usually correlated in cestodes (Kennedy 1983). Specific data from other cestodes were not available for comparison with the small r^2 (0.43) for correlation between mass and fecundity of T. crassus, but for mass vs. lifetime fecundity of rabbit ticks r^2= 0.64-0.84 (Campbell and Glines 1979), and for numbers of eggs in utero vs. mass in four species of intestinal nematodes r^2= ca.

0.65-0.88 (based on extrapolation from graphs in Ractliffe and LeJambre [1971]). There may in fact be a large genetic component to variability in T. crassus. This hypothesis should not be accepted by default through failure to recognize contributing environmental factors, but it is worthy of further consideration and critical testing.

OVERDISPERSION OF PARASITE NUMBERS

A striking feature of T. crassus populations in spawning pike was overdispersion or clumping of worm numbers among hosts, a common characteristic of parasitic infections (Anderson 1978; Anderson and Gordon 1982). In recent years the negative binomial distribution and its parameter k have frequently been used to quantify overdispersion but Esch (1983) cautions that fitting the distribution should not be an end unto itself, since a negative binomial distribution can arise through numerous mechanisms (Crofton 1971; Anderson 1976b; Arnason et al. 1986; Wassom et al. 1986). My study sampled only reproductively active hosts within a narrow time period, thereby reducing host heterogeneity relative to the population as a whole. Furthermore the parasite population was stratified into more restricted subsets of type 1, type 2, and type 3 worms (each presumably with narrower variability in age than worms in the population as a whole) thereby reducing the effect of temporally changing probabilities of infection as a source of overdispersion. Even within these subsets

overdispersion existed. Overdispersion within relatively uniform host and parasite populations may be attributable to individual variability in host feeding behavior (Anderson 1976b) or host resistance to infection (Arnason et al. 1986; Wassom et al. 1986). Complete refractiveness to infection by T. crassus probably does not occur since data from Southern Indian L. (chapter 2) showed all pike infected.

Overdispersion of parasites can potentially regulate parasite populations through death of heavily-infected hosts or intensity-dependent reduction in parasite fecundity (Anderson 1976a, 1978; Holmes et al. 1977; Kennedy 1977; Esch 1983). In my opinion there is no conclusive evidence for intensity-dependent regulation in populations of parasites in aquatic poikilothermic hosts, either from the literature or from my study on T. crassus. Consequently, overdispersion when intensity-dependent effects are minor does not lead to regulation but acts to concentrate a majority of the biotic potential of the parasite population into a few host individuals.

ECOLOGICAL INFLUENCES ON MATURATION OF T. CRASSUS

Triaenophorus crassus reproduces only once and does so within the narrow time frame of the host's spawning period. This synchronization results in deposition of parasite eggs in shallow water to facilitate transmission to copepods and then to coregonid fishes (Miller 1952; Lawler 1969). This is clearly advantageous for transmission and

natural selection should favor adaptations which maximize the probability that an individual worm will be fecund at the time of host spawning, and able to release its eggs. A widely accepted view (Michajlow 1933, 1962; Miller 1943a, 1952; Chubb 1963; Kuperman 1973) is that Triaenophorus spp. in general accomplish this within one year as worms are acquired in summer, mature over winter, and release their eggs the following spring. My study found that synchronization was well developed among the proportion of the T. crassus population that produced eggs; at least 70% were clearly ready to release their eggs during the spawning period. However, a majority of worms was unable to reproduce and this raises two questions: (1) Do these worms die after the spawning period, thus representing a major reduction in the reproductive potential of the population, or do they survive and mature over the following year? (2) Since T. crassus occurs in a wide range of north-temperate lakes possessing a variety of physical and biological characteristics, does the presence of immature worms in spawning pike reflect a fixed attribute of the parasite or does it reflect variations in ecological conditions between lakes? These questions have not been addressed in the literature but I believe that there is sufficient information available, from the literature on general aspects of the life cycle and from my study on individual variability of the parasite, to formulate a set of initial assumptions regarding ecological influences on the acquisition and maturation of T. crassus in northern pike, and formulate a model to determine whether these initial assumptions lead to predictions that are consistent with observations from field and laboratory studies. The following assumptions were made.

(1) Parasite recruitment is a function of the rate of host feeding and the proportion of infected hosts included in the prey.

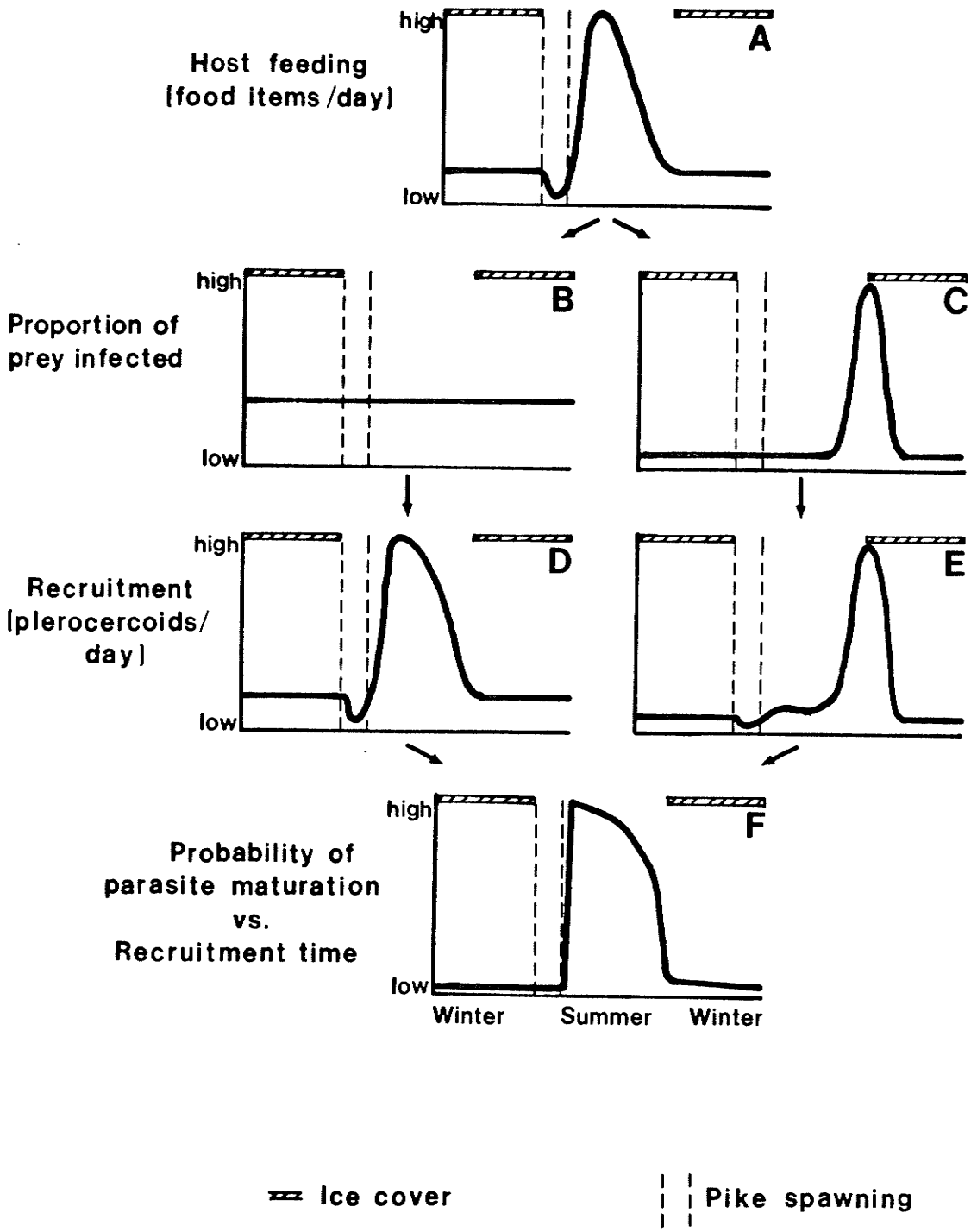
(2) All parasites are lost during host spawning time. No established worms die before that time. Several studies (Michajlow 1933; Miller 1943a; Chubb 1963; Kuperman 1973) report that numbers of Triaenophorus spp. are greatly reduced following spawning.

(3) The onset of maturation is independent of size (Roberts 1961; Kumazawa and Suzuki 1983).

(4) Seasonally-varying environmental conditions provide cues for parasite maturation. Temperature and host hormone levels are implicated in maturation of many cestodes (Esch 1983) and acanthocephalans (Amin 1978), and Libin (1951) found advanced gonadal development in T. crassus from pike given pituitary extract injections.

This model is based on seasonal changes in recruitment patterns and probabilities of parasite maturation. Feeding activity of pike varies seasonally; there is little overwinter feeding, accelerated feeding immediately post-spawning, and a decline in rate of feeding as the year progresses (Frost 1954; Diana 1979). This pattern is illustrated in Fig. 19A and its major features are probably similar between lakes. The proportion of infected hosts taken in the diet may be constant (Fig. 19B) or vary seasonally (Fig. 19C). Numerous patterns other than the two just described are possible and may depend on fish and lake characteristics. For example, small, shallow lakes with non-segregating fish species may be similar to Fig. 19B, while in deeper lakes pike and coregonids may be in contact only during limited periods. An example (Fig. 19C) shows pike preying on coregonids while

Figure 19. Hypothetical seasonal changes in recruitment of T. crassus by northern pike as a consequence of pike feeding behavior. A. Host feeding activity. B,C. Two possible seasonal patterns in the proportion of prey items infected with plerocercoids of T. crassus. D. Recruitment of T. crassus resulting from host feeding pattern (A) and prey selection pattern (B). E. Recruitment of T. crassus resulting from host feeding pattern (A) and prey selection pattern (C). F. Probability that plerocercoids acquired by pike at different times of year will mature and release eggs the following spring. See text for details.


Host feeding
(food items/day)
high
low
A
Proportion of
prey infected
B
C
Recruitment
(plerocercoids/
day)
D
E
Probability of
parasite maturation
vs.
Recruitment time
F
Winter
Summer
Winter
Ice cover
Pike spawning

the latter are on the spawning grounds as has been observed in Quigly L. (T.A. Dick, personal communication). Seasonal predation by pike on locally-abundant prey has been reported elsewhere (Frost 1954; Diana 1979). Under assumption 1 various predator-prey relationships could result in markedly different seasonal recruitment patterns (Fig. 19D,E), although in general recruitment would occur between spring and fall.

The probability that T. crassus recruited at different times of year will mature by the following spring is unknown, but if assumption (2) is true then parasites acquired in the immediate post-spawning period would have the highest probability since they would experience a maximum number of possible environmental cues for maturation and have the maximum time to complete maturation. This probability likely declines gradually over time because cues are missed and the remaining time decreases, and perhaps the probability declines more steeply as water temperatures drop and metabolic activities are slowed (Fig. 19F). Worms acquired late in the year would have virtually no possibility of maturing before host spawning and therefore die without reproducing. This raises the question as to what the composition of the T. crassus population at spawning time would be under different seasonal recruitment patterns and probabilities of maturation. Using the model various combinations of these can generate a wide range in the predicted proportions of mature worms as illustrated by the two extremes of predator-prey interactions in Fig. 19. If parasite recruitment followed the pattern in Fig. 19D then a high proportion of worms would mature by spawning time since they are acquired early when the probability of maturation is high. Some immatures would be

present due to low-level recruitment at other times of year. If on the other hand recruitment was as shown in Fig. 19E then most worms would be immature the following spring since recruitment was late. Few mature worms would be expected.

Data to test the model must include parasite recruitment patterns and proportions of mature worms in spawning pike. Suitable data for T. crassus came from Southern Indian L. (chapter 2). Pike fed heavily on cisco, particularly in the immediate postspawning period, and recruitment of T. crassus was shown to be completed by the end of June. The model predicts that most parasites would have matured since they were acquired at an early date. However, a March collection of pike had ca. 10% mature worms. The proportion of mature worms probably increased somewhat over the next two months but clearly most worms would still be immature at spawning time. One or more of the assumptions of the model would have to be violated to produce this result, and it is instructive to examine each of them. Three assumptions seem well supported. Recruitment patterns (assumption 1) were clearly established by stomach content analysis and changes in intensity of T. crassus within pike (chapter 2). Worms of all sizes seem capable of maturing (assumption 3) as shown by the range in sizes of type 3 worms at Falcon L. Assumption 4 would have been met at Southern Indian L. since all worms would have been exposed to a full range of seasonally-varying environmental conditions that might be a cue for maturation. The validity of assumption 5 is less clear. Although it was shown that there was no strong evidence to conclude that there were intensity-dependent effects on growth and maturation of T. crassus in natural infections (chapter 2), laboratory studies on

other cestodes have shown these to occur under conditions of nutrient limitation (reviewed by Read and Simmons [1963]).

The validity of assumption 2 is clearly questionable. Studies at Southern Indian L. did not find pike free of T. crassus in the spring (Watson 1977; chapter 2). The complete loss of T. crassus reported by Miller (1943a) was from a more southerly lake. A latitudinal cline may exist where worms in more northerly lakes require more than one year to complete the life cycle. Data on T. nodulosus indicates a higher proportion of immatures present farther north (Michajlow 1933; Chubb 1963; Borgstrom 1970; Kuperman 1973). It is unlikely that all immature T. crassus are able to persist as they outnumbered mature individuals at Southern Indian L. and Falcon L. and were therefore in excess of replacement requirements. In addition, the length-frequency histograms from mid-summer collections at Southern Indian L. (chapter 2) did not indicate the presence of a substantial cohort from the previous year's infection. Most immatures are probably expelled, even in northern lakes, but there may be a state of parasite development before which there is no response to the cue for detachment and these individuals may remain attached and mature over the next year. Rising spring temperatures are probably the cue for detachment (Kuperman and Shul'man 1972). Assumption 2 will be difficult to verify but should be possible with detailed seasonal studies on populations of T. crassus along a north-south cline and incorporating detailed studies of individual parasite characteristics to identify age cohorts of the parasite.

This model provides a simple framework in which lake-specific

predator-prey interactions can determine the proportion of T. crassus that are able to mature. Variations in worm age resulting from recruitment patterns, individual variations in growth rate, and independence of maturation from parasite size, would be expected to produce individuals of highly variable size and fecundity as was observed at Falcon L. Type 2 worms were not accounted for explicitly in the model, but the addition of a time lag between formation of eggs and the ability to release them would explain their presence. This initial application of the model suggests that the long-standing conclusion that T. crassus has an annual life cycle may not be generalizable to all lakes where this parasite occurs.

This model has interesting implications for the interpretation of parasite growth and reproductive characteristics from field data since it shows that worm maturation can be a function of acquisition time. Kennedy (1977) and Holmes et al. (1977) interpreted the presence of low proportions of gravid acanthocephalans in high-intensity infections as evidence for intensity-dependence. This may be true, but similar observations could result through variability in host feeding patterns over time relative to seasonal aspects of parasite maturation.

As with any model, the challenge for future studies is to use the model to test other host-parasite systems, particularly in north-temperate regions where parasites have a high degree of annual synchronization.

Parasites in general are considered to possess more r-selected than K-selected traits (Kennedy 1983), in particular high fecundity to

compensate for high juvenile mortality and for the low probability of successfully establishing and developing in a succession of hosts. If this is true why do we see large differences in fecundity even within a specific group of parasites such as tapeworms i.e. the observation that the population of T. crassus at Falcon L. remained stable in pike when lifetime parasite fecundity was ca. 120,000 eggs/worm while other tapeworms produce millions or tens of millions of eggs in their lifetime (Braten 1966; Kennedy 1983)? This question cannot be answered yet because many variables such as aquatic vs. terrestrial life cycles, poikilothermic vs. homeothermic hosts, pseudophyllidean vs. cyclophyllidean tapeworms, and semelparous vs. iteroparous reproductive patterns are involved, while fecundity estimates for most species are lacking. Perhaps the average fecundity of T. crassus can be lower than in other species since the absence of intensity-dependent effects in the natural population permits full expression of fecundity even in the presence of overdispersion of parasite numbers.

The presence of small, fecund T. crassus among larger, immature worms indicates that size is not a requirement for maturation of this species. Worms that grow but do not mature may have missed important cues for maturation. These individuals represent part of the "cost" of using a passive means for transmission of plerocercoids to the definitive host, and being unable to control time of acquisition, in a life cycle where there is great pressure for synchronization.

Of considerable interest is the effect of phenotypic plasticity in reproductive characteristics, and overdispersion of parasites among hosts, on transmission in a host-parasite relationship where

intensity-dependent effects are minimal. Behavior of the few heavily-infected pike harboring a majority of the biotic potential of T. crassus populations takes on added significance in transmission since the timing and location of spawning of those hosts will determine temporal and spatial distribution of parasite eggs in the aquatic environment. Furthermore, genetic diversity of their parasites is passed to the next generation rather than culled by parasite death through host mortality or by reduced parasite reproductive output. Individual hosts did not differentially alter phenotypic expression of size or fecundity in T. crassus, but since they provided the immediate environment during egg production they may influence egg quality. Consequently the few pike harboring the bulk of T. crassus and their eggs will not only influence parasite dispersal patterns but may have an enhanced role in transmission through effects on phenotype of the next parasite generation.

CHAPTER 4: VARIABILITY IN EGG HATCHING OF T. CRASSUS

ABSTRACT

During April of 1982-1984 eggs were collected separately from 178 Triaenophorus crassus obtained from northern pike at Falcon L., Manitoba. These eggs were maintained in dechlorinated water at a concentration of < 100 eggs/ml at 15C on a 12 h light: 12 h dark photoperiod and egg hatching was monitored over time. Three egg hatching characteristics were determined for each lineage (= eggs from the same adult worm): the proportion of eggs hatching (10-100%; mean across lineages= 74%), the median time from egg release to hatching (4.3-14.2 days; mean= 8.4 days), and the duration of the egg hatching period, expressed as the time between hatching of 10% and 90% of eggs in a lineage (1-17 days; mean= 4.7 days). Egg hatching characteristics were similar in lineages from the same host fish, most likely due to cues provided by a host's reproductive state. Egg dimensions were 51-79x35-49 μm (N= 439 eggs). Egg volumes varied significantly between individual worms within a fish but not between fish. Worms with fecundity < 100,000 eggs produced only small eggs, but a wide range of egg volumes with no clear relationship to fecundity was observed in more fecund worms. Egg volume was not related to the time taken by an egg to hatch after its release. It is suggested that variation within and between lineages of T. crassus in the duration between egg release and hatching is a mechanism to disperse coracidia of the parasite in time. This is considered either

an adaptation to environmental unpredictability or a method to minimize the possibility of high densities of coracidia producing lethal infection intensities in the copepod first intermediate host.

INTRODUCTION

The timing of egg release by parasitic helminths affects their dispersal in the environment and their probability of successful transmission to the next host. The cestode Triaenophorus crassus synchronizes its period of egg release with the spawning activities of its definitive host, the northern pike Esox lucius, and large numbers of parasite eggs are released in a location and at a time when copepods that serve as first intermediate host are abundant (Miller 1952; Lawler 1969; Smith 1973; Patrick 1984). The maintenance of synchrony is enhanced by long-term predictability of seasonal changes in environmental conditions and activities of the host species. By contrast, the environment of the eggs and the coracidia that hatch from them appears unpredictable in the short term. Temperatures in shallow water mixed by current and wind may vary with unpredictable short-term weather changes as illustrated by data from Falcon L. (chapter 3), and temperature affects development of the egg (Michajlow 1933; Watson 1963; Watson and Lawler 1963; Davydov and Strazhnik 1972; Kuperman 1973) and lifespan of the coracidium (Davydov and Strazhnik 1972). Copepods, although generally abundant in early spring, exhibit annual variations in the time of peak numbers (Smith 1973; Patrick 1984) and in some years their numbers decline earlier than in others (Smith 1973). The coracidium normally lives only a few days after hatching (Ekbaum 1937; Miller 1943b; Michajlow et al. 1971) and

therefore contact with a copepod must occur within a brief period. The importance of chance in this phase of the Triaenophorus spp. life cycle is illustrated by the observation of Watson and Lawler (1965) that although average prevalence of the parasite in 30,000 copepods they examined over a seven year period was only 1-2%, one sample of 85 copepods had 80 infected.

There are theoretical (Stearns 1976; Lacey et al. 1983; Kaplan and Cooper 1984) and empirical (Capinera 1979; Crump 1981; Wallace 1982; Lacey et al. 1983) reasons to suspect that organisms living in an unpredictable environment will have greater fitness if their offspring have high phenotypic plasticity in life-history characteristics. Many offspring will not survive because they are maladapted to immediate conditions, but the probability that no offspring survive is greatly reduced.

The presence of phenotypic plasticity in several aspects of the egg development and hatching process of Triaenophorus spp. is suggested in the data of several studies (Newton 1932; Michajlow 1933; Miller 1943b; Libin 1951; Kuperman 1973). Characteristics that vary among individuals are the proportion of eggs hatching, time when mass hatching occurs, and duration between hatching of the first and last eggs. In addition, egg sizes of T. crassus vary among populations (Newton 1932; Ekbaum 1937; Miller 1943b; Lawler and Watson 1963; Kuperman 1973). However, apart from suggesting the presence of phenotypic variability, the available data do not permit the extent of individual variability to be quantified or the sources of this variability to be identified.

In this study an individual cestode and all its descendants will be termed a lineage. The term hatching refers to the exit of a coracidium from an egg, and hatch time refers to the duration of the interval between release of an egg by an adult worm, and egg hatching. "Hatching characteristics" is used as a collective term for the proportion of eggs hatching, the median hatch time, and variation in hatch time of individual eggs within a lineage.

The objective of this study was: (1) to assess variability in egg morphology and hatching characteristics of eggs obtained from adult T. crassus of known origin and maintained under controlled conditions, and (2) to consider the role of this variability in temporal dispersal of the coracidium.

DATA COLLECTION

Eggs were collected from individually-identified T. crassus during 1982-1984 according to the methods in chapter 3. The day an adult worm was collected and first released eggs was day 0. All water used was dechlorinated and aerated.

The dimensions of unembryonated eggs were estimated from photographs of wet mounts, taken with a Zeiss Photomicroscope II, within 6 h of release from the uterus of type 3 worms (chapter 3) in the 1984 sample. A stage micrometer was photographed for calibration. Length and width of up to 10 eggs in a lineage were measured on the negative, and volume estimates made by assuming eggs have an ellipsoid shape.

Eggs from each lineage were prepared for hatching observations on day 1. Eggs were stirred using a magnetic stirrer and aliquots of the egg suspension removed and counted. Sufficient volume of suspension to ensure ca. 100 eggs/sample was removed and placed in each of 20, 2

ml flat-bottomed polystyrene Autoanalyzer sample cups (Fisher Scientific, Winnipeg, Manitoba). Water volume was raised to 2 ml and all sample cups were stored at 14.5-15.5C under a 12 h light: 12 h dark photoperiod. Water levels were maintained at 1.5-2 ml; additions were required every 2-3 days.

Every 1-2 days, starting on day 3, one sample cup was selected at random from each lineage for observation. Observations did not commence until at least 1 h after lights were turned on. The water column was scanned at 40x using a dissecting microscope to count numbers of floating eggs and record presence of coracidia. Most of the water in each vial (1.8 ml) was pipetted off leaving eggs in a water film on the bottom of the sample cup. The cup was then placed on a gridded glass slide and scanned at 40x to count numbers of hatched and unhatched eggs. Empty eggs were not present in newly-prepared samples so their presence in later samples indicated that a hatch had occurred. Daily observations were continued on each lineage until hatching of eggs was completed.

DATA ANALYSIS

Plots of the proportion of hatched eggs vs. time were sigmoid in shape, but in most lineages the asymptote did not reach 100% but fluctuated around a lower level indicating that some eggs were unable to hatch. This proportion was estimated graphically by fitting a line by eye through data in the asymptote region, such that no more than

one data point in that region was more that five percentage points from the line. In ca. 5% of lineages there was excessive random scatter of data points and this criterion could not be met; those lineages were not used.

The hatching curve for each lineage was analyzed using a maximum likelihood PROBIT procedure in SAS. Numbers of hatched and unhatched eggs (after correcting for the proportion of eggs unable to hatch) were used as response variables, and time transformed by ln(days) was used as the dose variable. Residual plots were examined and if systematic departures were present the analysis was not used. The estimated median hatch time (LD50 or ED50 of common usage) and the variance about the median time were used in subsequent analyses as indicators of the duration between egg release and mass hatching, and of the rate of hatching during that time, respectively. A lineage that had a similar median hatch time to another, but took longer for all eggs to hatch, would have a higher variance.

A nested analysis of variance (ANOVA) was used to identify sources of variability in egg volume and egg hatching characteristics, using the procedures outlined in chapter 3. A coefficient of variation was calculated as $100 \times SD/\bar{X}$. Product-moment correlations were used unless specified otherwise. Statistical significance was determined using $\alpha = 0.05$.

RESULTS

Up to 10 eggs from each of 47 T. crassus were measured. Mean egg dimensions, and minimum and maximum values, were with one exception within the range reported in other studies (Table 24). The maximum egg length, 79 µm, was greater than reported elsewhere. The worm that released this egg also had other long eggs, but contamination can be ruled out since gravid worms of other species (with eggs that might be mistaken for T. crassus) were not present in the same host fish. The distribution of egg volumes was wide and skewed right (Fig. 20) but most eggs fell within a narrow range, ca. 0.040-0.055 nl. Egg volumes differed significantly between worms attached within the same section of the host's intestine (see chapter 3 for definition of section), and between worms attached in different sections of a host fish (Table 25). The variability of egg volumes within a worm was the error variance, and was only about one-quarter of total variance in egg volume (Table 25). The coefficients of variation for eggs within a worm were 4-21% but most were 8-14%.

Fecundity estimates were available for all lineages (chapter 3). Egg volume tended to be greater in more fecund worms (Spearman rank correlation: $r = 0.298$; $N = 45$; $P = 0.047$) but the relationship was complex (Fig. 21). Worms with fecundity less than ca. 100,000 eggs produced only small eggs, while worms with higher fecundity produced

Table 24. Egg sizes of T. crassus.

Location	N	Length, µm	Width, µm	$\bar{X}$ volume, nl
Falcon L., 1984	439	60.6 (51-79)[f]	40.4 (35-49)	0.053
Manitoba, ca. 1932[a]	2?	64.5 (64-65)	40 (-)	0.054
Heming L., 1953[b]	53	57.1 (48-65)	36.6 (31-43)	0.040
" , 1957[b]	7	57.6 (55-61)	37.4 (36-38)	0.042
Lesser Slave L., 1943[c]	-	61 (53-68)	41 (38-44)	0.054
Ontario, 1935[d]	-	58 (54-64)	40 (36-44)	0.049
L. Huron, 1958[b]	101	58.1 (47-66)	37.9 (32-43)	0.044
Rybinsk res., -[e]	42	65 (44-72)	41.5 (38-50)	0.059

[a] Newton (1932)

[b] Lawler and Watson (1963)

[c] Miller (1943b)

[d] Ekbaum (1937)

[e] Kuperman (1973)

[f] $\bar{X}$ (range)

Figure 20. Frequency distribution of egg volumes of T. crassus from Falcon Lake, Manitoba. UCL, upper class limit.

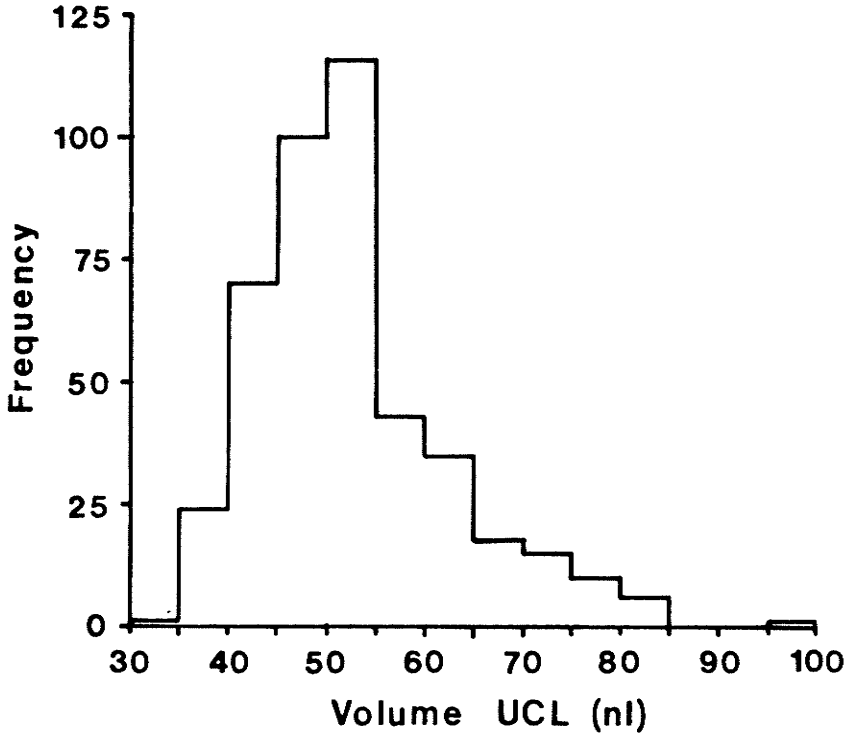
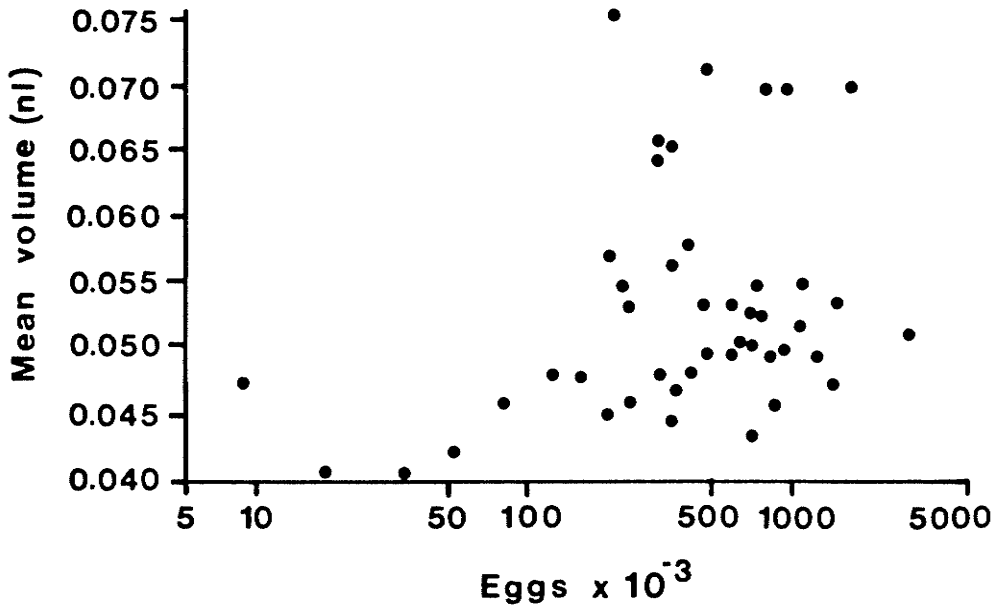

125
100
75
50
25
0
Frequency
30
40
50
60
70
80
90
100
Volume UCL (nl)

Table 25. Nested analysis of variance on volumes of eggs from *T. crassus* collected in 1984. Data were transformed by ln(n1+1).

Source	df	MS	% Variance component	Significance test[a]
Fish	6	0.66524	10.46	F'=1.430; df= 6, 8; P= 0.311
Section	10	0.35596	36.11	F'= 3.758; df= 10, 29; P= 0.003
Lineage	30	0.09445	26.26	F= 10.02; df= 30, 392; P< 0.001
Error	392	0.00943	27.17	
Total	438	0.03215	100.00	

[a] F' is approximate test using Satterthwaite approximation.

Figure 21. Mean volume per egg from T. crassus with various fecundities. Means are based on at least five eggs.


0.075
0.070
0.065
0.060
0.055
0.050
0.045
0.040
Mean volume (nl)
5
10
50
100
500
1000
5000
Eggs x 10-3

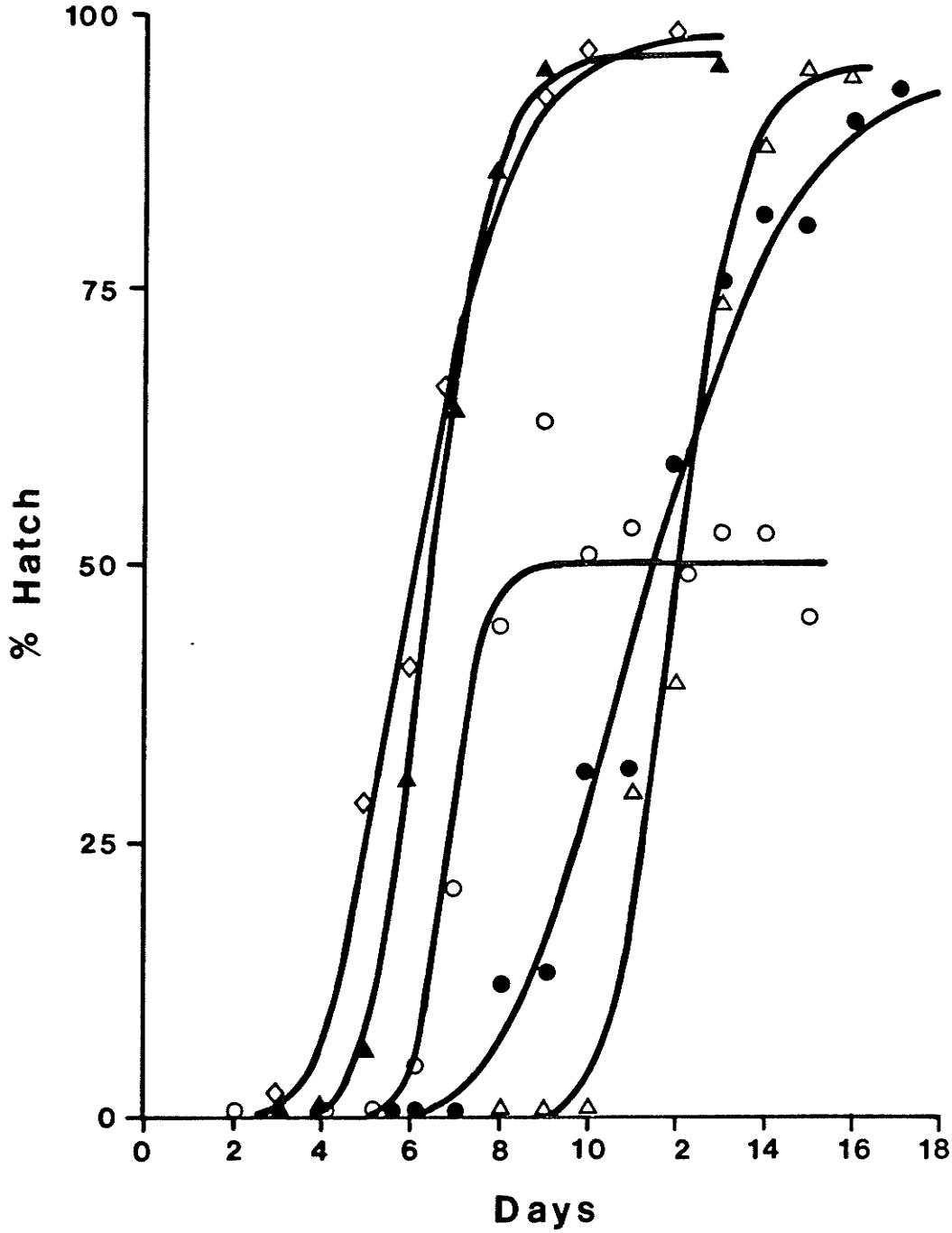
eggs having a wide range of volumes.

The pattern of egg hatching over time varied between lineages, but was always sigmoid in form. Several examples (Fig. 22) illustrate that patterns were distinguishable on three characteristics: (1) the proportion of eggs hatching, (2) the median hatch time (the day on which eggs within a lineage hatched at the greatest rate, occurring at the steepest portion of the curve), and (3) the variance of hatch time among eggs within a lineage (indicated by the spread of the curve along the time axis). Probit analysis on data adjusted for non-hatching eggs provided estimates for (2) and (3) above, respectively termed the median egg hatch time and the variance in egg hatch time. These two estimates were not correlated ($r= 0.045$; $N= 179$; $P= 0.554$). The widths of the 95% fiducial limits for median egg hatch times varied with the median and its variance, but usually were within $\pm$ 1 day of the median.

The proportion of eggs hatching (transformed by arcsin $\sqrt{p}$) was positively correlated with median egg hatch time ($r= 0.254$; $N= 179$; $P< 0.001$) and was negatively correlated with variance about the median hatch time ($r= -0.262$; $N= 179$; $P< 0.001$), but the low r^2 in both relationships indicated that most of the variation of these variables was not explained by the magnitude of the other. In the remaining analyses the proportion of eggs hatching, median egg hatch time, and variance about the median, were treated as independent characteristics of egg hatching in T. crassus.

Median egg hatch time did not vary significantly with fecundity, but the variance in egg hatch time and proportion of eggs hatching

Figure 22. Percentage of eggs of T. crassus hatched at various times after release from the adult worm. Eggs from five lineages are shown, indicated by different symbols.


100
75
50
25
0
% Hatch
0
2
4
6
8
10
2
14
16
18
Days

were significantly greater in more fecund worms (Table 26). The median egg hatch time and its variance were significantly greater in worms with larger mean egg volumes, but variance in egg hatch times was not correlated with variance in egg volumes within a lineage as measured by the coefficient of variation (Table 26).

Variation in the proportion of eggs hatching was primarily attributable to variations between host fish or year of collection (Table 27A). Proportions hatching were 66 $\pm$ 17% in 1982 (N=59), 84 $\pm$ 17% in 1983 (N=53), and 94 $\pm$ 8% in 1984 (N= 69). The error term, which was variation among worms attached within a section of the intestine, and variation among worms from different sections of the intestine within a fish, accounted for only about one-third of total variance (Table 27A). A high proportion of eggs hatched in most lineages (Fig. 23A); hatch of fewer than 50% of eggs occurred in only 9% of the lineages.

Variation in the median time of egg hatching was primarily attributable to variations between fish or year of collection (Table 28A) but in contrast to the proportion of eggs hatching (Table 27A), added variance in hatch time due to year was only marginally significant. Median egg hatch time was 7.6 $\pm$ 2.0 days in 1982 (N= 58), 9.4 $\pm$ 1.5 days in 1983 (N= 51), and 8.3 $\pm$ 1.6 days in 1984 (N= 67). Median egg hatch times varied from 4.3-14.2 days and had an approximately symmetrical frequency distribution with most values between 6-10 days (Fig. 23B).

Variation in the variance of egg hatch times within a lineage was attributable to host fish and year of collection (Table 29). This

Table 26. Correlations between fecundity, mean egg volume per worm, and coefficient of variation (CV) in egg volume of T. crassus with hatching characteristics of the eggs.

Hatching characteristics		Fecundity[a]	$\bar{X}$ volume[a]	CV
Median hatch time	r=	-0.083	0.322	0.025
	N=	176	45	41
	P=	0.273	0.031	0.876
Variance of hatch time	r=	0.216	0.327	-0.250
	N=	176	43	39
	P=	0.004	0.032	0.128
Proportion hatching[b]	r=	0.222	0.187	0.068
	N=	178	45	41
	P=	0.003	0.217	0.674

[a] transformed by ln(x)

[b] transformed by arcsin $\sqrt{p}$

Table 27. Nested analysis of variance on proportions of eggs of T. crassus that hatched. Data were transformed by arcsin $\sqrt{P}$. A. Eggs from all worms included. B. Only eggs from lineages used in chapter 7.

Source	df	MS	% Variance component	Significance test[a]
A.				
Year	2	2.52674	54.77	F'= 23.76; df= 2, 15; P< 0.001
Fish	22	0.06821	10.08	F'= 3.487; df= 22, 16; P= 0.007
Section	36	0.02127	0.00	F= 0.805; df= 36, 117; P=0.770
Error	117	0.02641	35.16	
Total	177	0.05881	100.00	
B.				
Year	1	0.17250	9.89	F'= 1.649; df= 1, 9; P= 0.230
Fish	11	0.06657	62.09	F'= 10.01; df= 11, 7; P= 0.003
Section	10	0.00694	0.00	F= 0.681; df= 10, 15; P= 0.727
Error	15	0.01019	28.02	
Total	37	0.03050	100.00	

[a] F' is approximate test using Satterthwaite approximation.

Figure 23. Frequency distributions of hatching characteristics of eggs from different lineages of T. crassus. A. Percentage of eggs able to hatch. B. Median times between release of eggs of different lineages, and egg hatching, as determined by probit analysis. Solid bars, distributions for lineages used in the analyses of chapter 7.

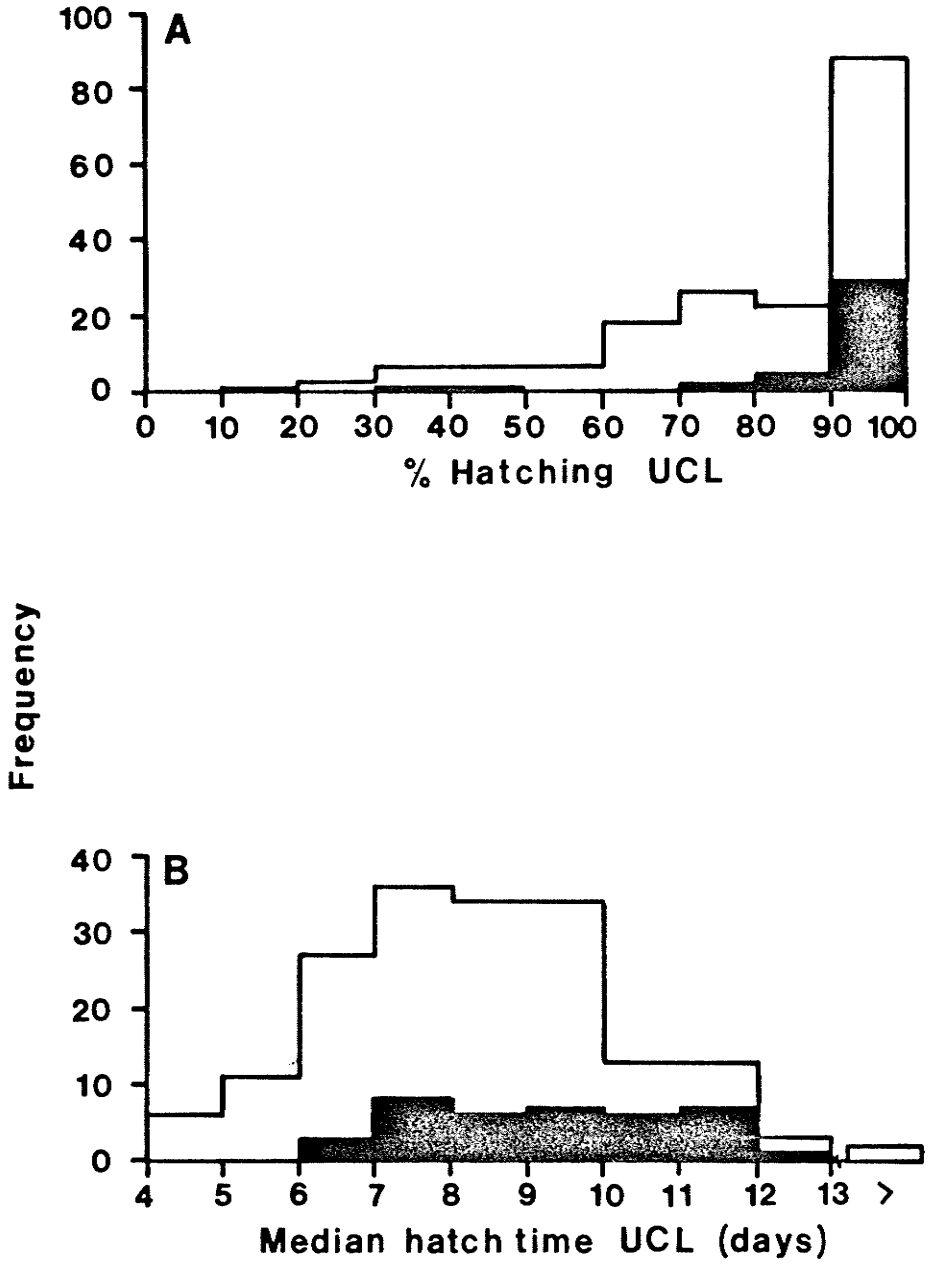

A
100
80
60
40
20
0
0 10 20 30 40 50 60 70 80 90 100
% Hatching UCL
Frequency
B
40
30
20
10
0
4 5 6 7 8 9 10 11 12 13 >
Median hatch time UCL (days)

Table 28. Nested analysis of variance on median time of egg hatching (ln days) of T. crassus. A. Eggs from all worms included. B. Only eggs from lineages used in chapter 7.

Source	df	MS	% Variance component	Significance test[a]
A.				
Year	2	0.78897	14.01	F'= 2.568; df= 2, 19; P= 0.102
Fish	22	0.17274	42.34	F'= 12.14; df= 22, 13; P< 0.001
Section	36	0.01702	0.00	F= 0.660; df= 36, 115; P= 0.923
Error	115	0.02577	43.64	
Total	175	0.05117	100.00	
B.				
Year	1	0.08741	0.00	F'= 0.707; df= 1, 10; P= 0.424
Fish	11	0.07830	73.10	F'= 9.623; df= 11, 8; P= 0.002
Section	10	0.00827	0.00	F= 0.850; df= 10, 15; P= 0.593
Error	15	0.00973	26.90	
Total	37	0.03182	100.00	

[a] F' is approximate test using Satterthwaite approximation.

Table 29. Nested analysis of variance on the variance of median time of egg hatching of T. crassus. A. Eggs from all worms included. B. Only eggs from lineages used in chapter 7.

Source	df	MS	% Variance component	Significance test[a]
A.				
Year	2	0.30001	26.11	F'= 5.409; df= 2, 17; P= 0.015
Fish	22	0.03269	26.03	F'= 5.320; df= 22, 17; P= 0.001
Section	36	0.00652	0.00	F= 0.847; df= 36, 115; P= 0.711
Error	115	0.00770	47.86	
Total	175	0.01394	100.00	
B.				
Year	1	0.02372	12.96	F'= 3.479; df= 1, 6; P= 0.110
Fish	11	0.00529	19.19	F'= 2.874; df= 11, 6; P= 0.104
Section	10	0.00207	0.00	F= 0.452; df= 10, 15; P= 0.896
Error	15	0.00458	67.84	
Total	37	0.00462	100.00	

[a] F' is approximate test using Satterthwaite approximation.

variance, measured as ln(days), was 0.29 ± 0.15 in 1982 (N=59), 0.20 ± 0.08 in 1983 (N=53), and 0.16 ± 0.07 in 1984 (N= 67). Collectively these accounted for about half of the variation between lineages. The frequency distribution for variance of egg hatch times was skewed right (Fig. 24A) indicating that a majority of lineages tended to hatch over a brief period and in only a few lineages was the egg hatching period protracted. Since variance about the median egg hatch time was calculated on a logarithmic time scale it cannot be directly converted to indicate the duration of the egg hatching period in days without knowledge of the median egg hatch time. In addition, this duration ranges in theory from 0 days to infinity and its empirical determination is sample-size dependent (Finney 1971). As a means of directly comparing the duration of the egg hatching period among lineages, I chose the time period over which the central 80% of eggs hatched (from 10-90%), calculated for each lineage from a probit analysis. Over the range of median hatch time in this study there was a high correlation between the duration of the egg hatching period (in days) and the variance about the median egg hatch time (in ln[days]) (Spearman correlation: r= 0.894; N= 176; P< 0.001). The number of days coracidia were present in the water column was also higher in lineages with larger variances in egg hatch times, providing independent evidence that lineages differed in the duration of the egg hatching period. Nested ANOVA on the duration of the egg hatching period (Table 30) produced similar results to those from nested ANOVA on variance in egg hatch times (Table 29), although added variance due to year was not significant (Table 30). The eggs from most lineages completed the central 80% of their hatching over a period of 2-5 days

Figure 24. Frequency distributions of two measures of variability in egg hatching times within a lineage of T. crassus. A. Variance in egg hatch time, as determined by probit analysis. B. Duration between 10% hatch and 90% hatch of eggs, rounded to the nearest day. Solid bars, distribution for lineages used in the analyses of chapter 7. UCL, upper class limit.

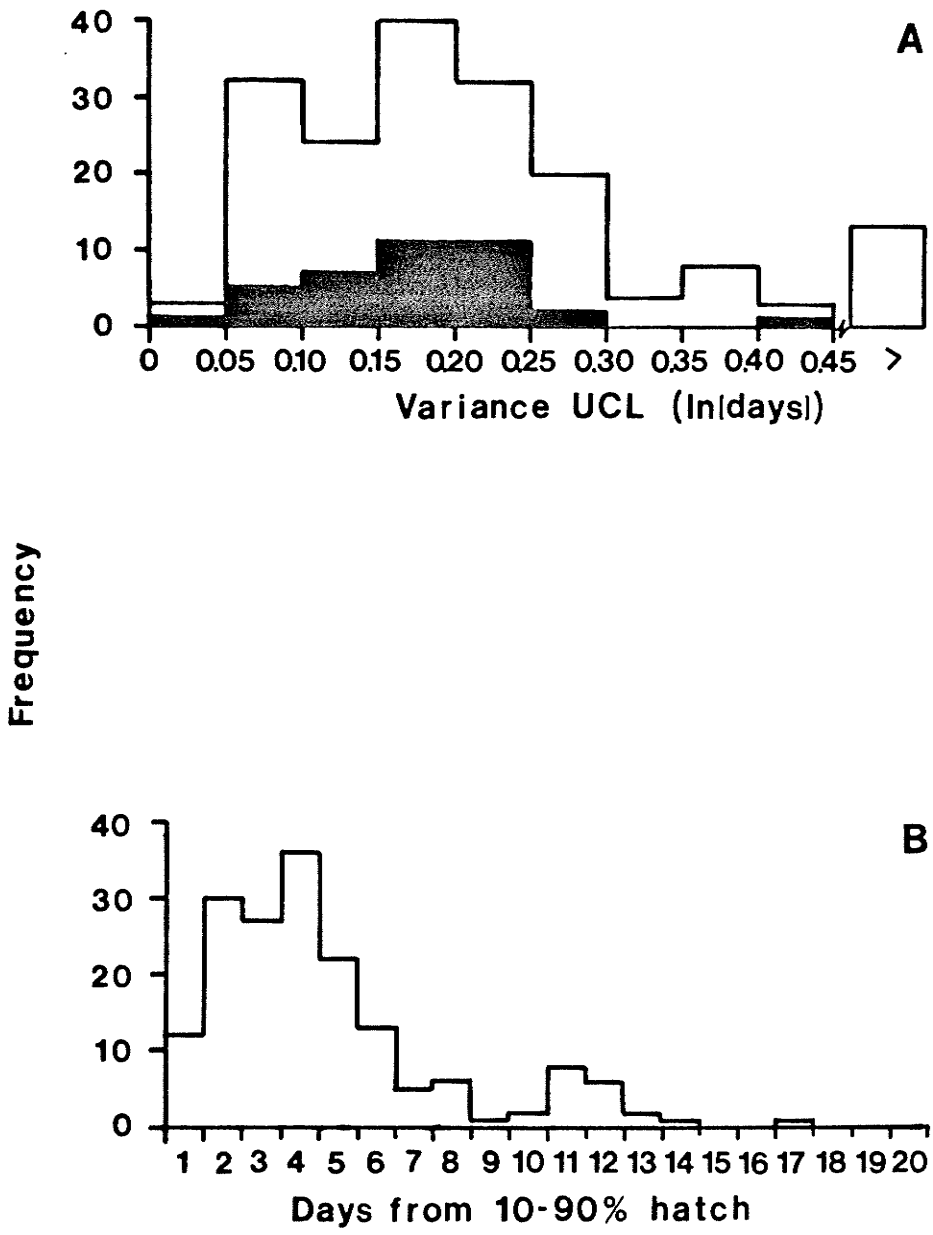

A
40
30
20
10
0
0 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45 >
Variance UCL (ln[days])
Frequency
B
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Days from 10-90% hatch

Table 30. Nested analysis of variance on the duration between 10% hatch and 90% hatch of eggs of T. crassus. Data were transformed by ln(days).

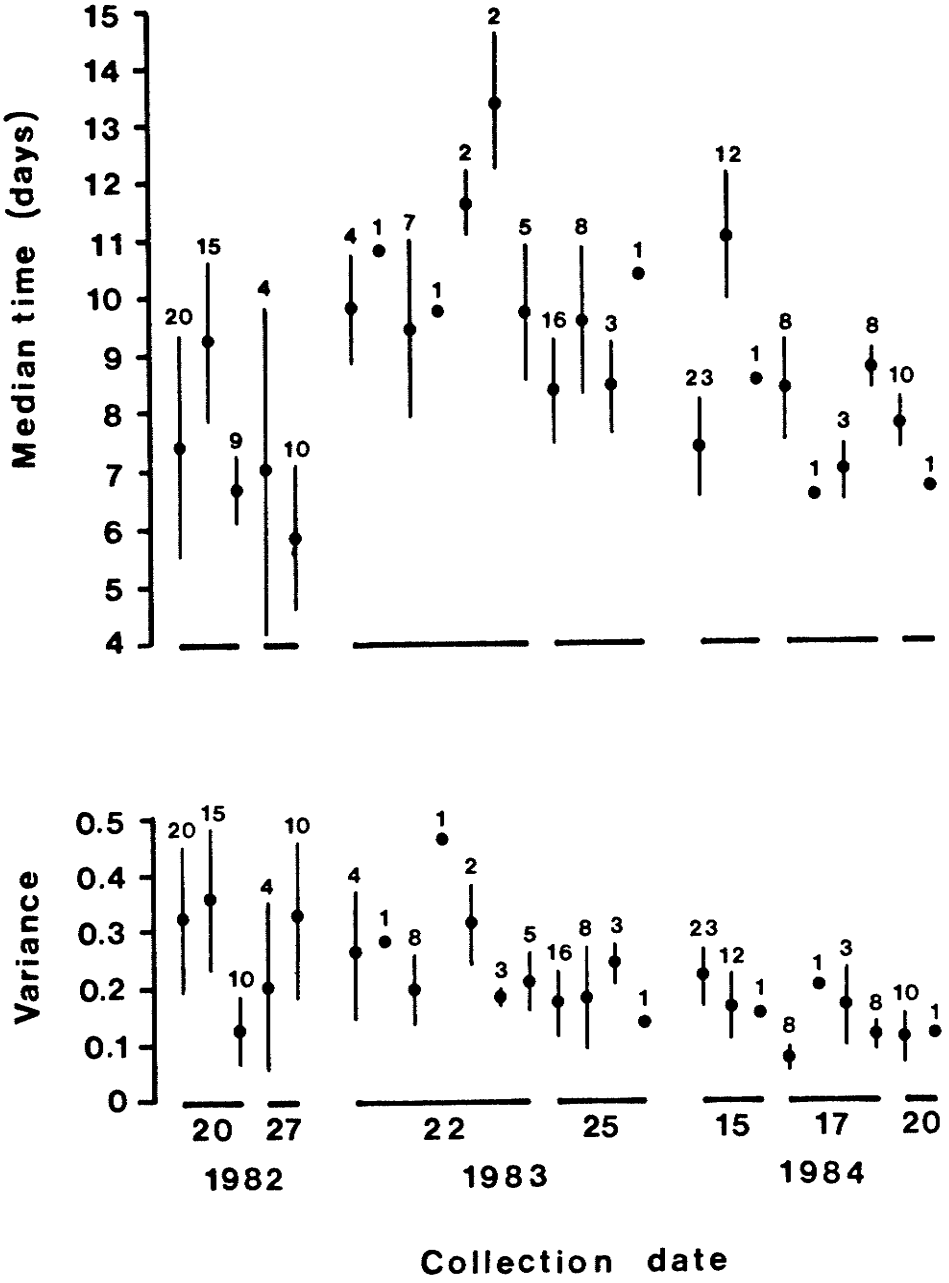
Source	df	MS	% Variance component	Significance test[a]
Year	2	5.35946	11.13	F'= 2.103; df= 2, 19; P= 0.148
Fish	22	1.41338	44.29	F'= 7.116; df= 22, 20; P< 0.001
Section	35	0.19805	0.17	F= 1.010; df= 35, 113; P= 0.467
Error	113	0.19618	44.40	
Total	172	0.41228	100.00	

[a] F' is approximate test using Satterthwaite approximation.

but in several lineages hatching was highly synchronized and 80% of eggs hatched within a 1 day period (Fig. 24B). Many lineages took about 2 wk to complete 80% of egg hatching (Fig. 24B).

Previous analyses indicated that hatching characteristics of the eggs of T. crassus varied significantly among fish from which the lineages originated. Data from each fish were plotted relative to their dates of collection (Fig. 25) to determine if day of collection within a year was a cause. There was wide inter-fish variation within each day of collection. In a preliminary analysis (not shown) < 0.01% of total variance in median egg hatch time and its variance were attributable to the relative day of collection of fish within a year (i.e. early, middle, or late in the spawning run). In addition, egg hatching characteristics did not seem to be related to the absolute date of collection, which varied from 15 April- 27 April over three years (Fig. 25).

Figure 25. Variability in egg hatching characteristics of T. crassus from different northern pike. Each point is $\bar{X} \pm SD$ of median hatch time or variance in hatch time for eggs from all T. crassus within one pike. N, number of lineages of T. crassus within each pike. Collection dates are all in April of the year indicated.


Median time (days)
15
14
13
12
11
10
9
8
7
6
5
4
20
15
9
4
10
4
1
7
1
2
2
5
16
8
3
1
23
12
1
8
1
3
8
10
1
Variance
0.5
0.4
0.3
0.2
0.1
0
20
15
10
4
10
4
1
8
1
2
3
5
16
8
3
1
23
12
1
8
1
3
8
10
1
20
27
22
25
15
17
20
1982
1983
1984
Collection date

DISCUSSION

The life cycle of T. crassus has mechanisms to synchronize release of eggs by all worms in the host population, yet also has mechanisms to promote dispersal in time of coracidia that hatch from those eggs. Previous studies suggested that hatch times of T. crassus are variable (Newton 1932; Miller 1943b; Kuperman 1973) but did not address the magnitude or causes of that variability. My controlled experiments allowed quantification of the extent and causes of this phenotypic variability, and enabled me to speculate on the role of individual variability in parasite transmission.

There are two major factors that affect phenotypic variability: intrinsic factors which are the expression of a species' genotype, and environmental factors which for a parasite species include both the abiotic environment and the environment provided by the host. Contributions of genotype to variability were not directly evaluated, but were assumed to be included within variability that could not be directly accounted for through environmental effects. This study was primarily designed to examine the host fish as an environmental source of phenotypic variability in the hatching characteristics of parasite eggs, but egg volume estimates provided a means of assessing the relationship between adult phenotypic variability and phenotypic variability in the hatching characteristics of their offspring. The

volume of eggs presumably reflects maternal phenotypic variability in size of oocytes and the structure of the oogenotop in which yolk is laid down and egg shell formation occurs (Schmidt and Roberts 1977), and egg volume may be a source of phenotypic variability in hatching characteristics (Crofton and Whitlock 1965a,b).

Physical factors known to affect development and hatching of eggs are temperature (Watson and Lawler 1963; Kuperman 1973), light (Kuperman 1973; Macdonald 1975), and oxygen (Pojmanska 1957; Watson and Lawler 1963; Michajlow et al. 1971). Development of eggs prior to their time of collection in this study was presumably influenced by temperature, but since eggs develop over winter (Miller 1943a) and water temperatures in an ice-covered lake vary minimally, temperature effects prior to egg collection were probably uniform on the T. crassus in Falcon L. Differences in the intestinal environment may have occurred among pike, but physical conditions after egg collection were controlled, thereby removing much of their potential effect on phenotype.

The environment of each worm was treated as a hierarchy of levels i.e. lineages from adult worms attached in the same intestinal section, different sections within a fish, different fish within a year, and different years. At the lowest level, which was variation among worms within a section, causes of variation were indeterminate. This was error variability and included: (1) measurement error, (2) unidentified or unmeasurable environmental effects on phenotype (such as age of plerocercoids that initially infected pike, or other environmentally-induced parental variability that affected egg

formation), and (3) genetic differences among worms. Error variance components were low (27-48%) in comparison with error components for mass and fecundity of adult worms as determined in the previous chapter using a similar environmental hierarchy for analysis. Allowing for sources (1) and (2), the genetic component was probably lower than the total error variability component.

The next hierarchical level was worms attached in different intestinal sections within a fish. As discussed in chapter 3, worms in different sections probably differed in age more than worms within a section. No variability among sections was detected in three hatching characteristics (proportion hatching, median and variance of hatch times) but there was inter-section variability in egg volume. This contrasts with the next higher level in the hierarchy, variations among fish, in which the three hatching characteristics listed above differed significantly but egg volumes did not. This suggested some independence in the processes of egg formation and development, but more interestingly it indicated that there was uniformity of hatching characteristics of eggs from all worms within a fish. This uniformity within a host may be due to some attribute of the host fish which induced similar phenotypic changes in development of all eggs, or to an increase in homozygosity resulting from low numbers (1-29) of sexually-mature worms mating within each infrapopulation. There was some evidence for the first possibility. Maturation in T. crassus is influenced by host hormone levels (Libin 1951) and individual variability in timing of maturation among pike may be sufficient to cause variability in parasite egg hatching characteristics between host fish but uniformity within. There was no conclusive evidence for

or against the second possibility, although if hatching characteristics were heritable and there were no host-induced phenotypic effects then mean hatching characteristics of eggs from hosts with larger breeding populations of T. crassus should be similar among hosts. Examination of Fig. 25 suggests this was not the case. An experimental approach is required to resolve whether one or a combination of these two mechanisms is responsible for the uniformity of egg hatching characteristcs within a host. Although the causative mechanism is not clear, the presence of this uniformity may have considerable importance in parasite transmission, and will be discussed shortly.

The highest level of the environmental hierarchy was year of collection. This level was responsible for a majority of observed variability in the proportion of eggs hatching, and for small but significant inter-lineage differences in the variance of egg hatch times. Possible causes of year-to-year variations may be variations in physical conditions such as temperature or light that may affect the parasite directly or indirectly through the host, or differences in the biotic environment such as predator-prey interactions that determine parasite age structure or perhaps recruitment by a particular year class of plerocercoids with unique biological characteristics. Variations resulting from experimental procedures may have occurred between years, although experiments were carefully controlled regarding materials and protocols.

Egg dimensions of T. crassus show a small but similar range of variation in all populations examined (Table 24) suggesting that there

are fairly rigid, species-wide constraints on the process of egg formation. However, within that range there was variability in egg volumes within and between adult worms. Large eggs may take longer to hatch (Crofton and Whitlock 1965a,b) suggesting that egg volume variability was a possible cause of variability in hatching characteristics of eggs of T. crassus. Mean egg volumes and median egg hatch times of T. crassus were positively correlated, but this did not indicate cause-and-effect since variance of individual hatch times was not greater in lineages with more variable egg sizes. Furthermore, variability in egg volumes from worms in different host fish was low while differences in egg hatching characteristics varied markedly between fish. Consequently, for T. crassus the factors influencing egg formation were independent of factors influencing egg hatching characteristics.

This study established two characteristics of egg hatching in T. crassus that can affect dispersal of coracidia in space and time. First, there was wide inter-lineage phenotypic variability in median hatch time and duration of the egg-hatching period. The values obtained were in agreement with the more qualitative results of other studies on Triaenophorus spp. (Newton 1932; Ekbaum 1937; Miller 1943b; Libin 1951; Pojmanska 1957; Watson and Lawler 1963; Kuperman 1973). Because of this variability coracidia of the same adult worm will be dispersed over several days to two weeks, and peak numbers of coracidia of different worms will be dispersed over a similar time frame. Complete synchronization of hatching within a lineage (80% or more hatching within a 1-day period) was rare. Second, the relative uniformity of egg hatching characteristics among T. crassus within a

host suggests that host behavior is also important in dispersal of coracidia on the spawning grounds, particularly when numbers of the parasite are overdispersed among pike. What are the advantages of phenotypic plasticity in egg hatching times when there seems to have been strong selective pressure for general synchronization of egg release within the parasite population? Is it an adaptation to an unpredicatable environment or is it an adaptation to minimize loss of parasites through mortality of the copepod host?

Phenotypic variability of offspring is suggested to be an adaptation to unpredictable environments by increasing the probability that at least some offspring have an appropriate phenotype for the conditions they encounter (Stearns 1976; Capinera 1979; Crump 1981; Wallace 1982; Lacey et al. 1983; Kaplan and Cooper 1984). In assessing environmental variability it is important to consider factors pertinent to the organism being studied (Crump 1981; Lacey et al. 1983). For coracidia of T. crassus physical conditions and the copepod host are critical factors. Unpredictable, short term changes in the physical environment probably occur. Coracidia are susceptible to high temperatures (Davydov and Strazhnik 1972) such as may occur in shallow water on an unusually hot day. Wind or wave action from a chance storm may disperse coracidia to unsuitable locations. Copepod abundances vary within and between years (Smith 1973; Patrick 1984), and local abundances vary within and between days as a function of vertical migration (Patrick 1984). Random annual variations in the arrival of pike on the spawning grounds relative to periods of peak copepod abundance could in some years favor eggs hatching soon after release, while in other years favor eggs that do not hatch for a week

or more. Random annual variations and unpredictable short term changes in the environment of the short-lived coracidium cannot be discounted as selective forces for phenotypic vaiability in egg hatching of T. crassus. The failure of all offspring of an annual parasite to survive is catastrophic.

As far as I am aware the role of variability in egg hatch times as an adaptation to reduce the frequency of multiple infections in the copepod host under natural conditons has not been considered, possibly because low prevalences and intensities are usually observed in natural infections (Watson and Lawler 1965; Sysoev 1981; Esch 1983). However, in view of the high fecundity of T. crassus (determined in chapter 3) and increased host mortality in multiple infections of the copepod host (Rosen and Dick 1983) this possibility warrants consideration, particularly given the number of parasite eggs that can be released within a relatively brief period. For example, if 15,000,000 eggs of T. crassus were released from a single host fish (chapter 3) over an area 500 m^2 in water 1 m deep and hatched synchronously, a density of 30 coracidia/l would result. Actual densities could be much higher due to egg release from other pike in the area. Cyclops bicuspidatus thomasi, the host of T. crassus, is a selective feeder on soft-bodied prey (Stemberger 1985) and readily ingests coracidia (Michajlow 1962). Miller (1943b) noted that C. b. thomasi can eat 4-5 coracidia of T. crassus within one hour, and Mesocyclops edax can consume 4 prey/day at a density of 30 prey/l (Williamson 1984). I conclude that under natural conditions densities of coracidia capable of causing multiple infections of the copepod host may occur if all eggs hatch synchronously. Dispersal of

coracidia in time through phenotypic variability in hatch times of the eggs (within and between lineages) will substantially reduce their density yet maintain their presence within the period that the copepod host is abundant in the environment.

This study found a complex pattern of phenotypic variation in the eggs and egg hatching characteristics of T. crassus. The extent to which this reflected underlying genetic diversity was not determined, although variability that was not explained by identifiable environmental factors was low. This was not surprising, since a large component of environmentally induced variability is expected for a parasite that is critically dependent on its fish-host environment to cue its own reproductive activities. Regardless of the causes of phenotypic variability in egg hatching characteristics, the effect is a dispersal of the free living coracidia in time. It is surprising for a semelparous parasite such as T. crassus that virtually nothing is known empirically regarding the distributional pattern of its eggs on the spawning grounds or the relationship between densities of coracidia and copepods during the critical transmission period. Phenotypic variability causing dispersal of coracidia in time, on the short-term time scale relevant to the free-living stages, may be a critical adaptation of a basically synchronized life cycle to deal with environmental uncertainty and perhaps to reduce the probability of copepod, and consequently parasite, mortality.

CHAPTER 5: COPEPOD - PROCERCOID NUTRITIONAL INTERACTIONS

ABSTRACT

Host fecundity, and parasite growth and development, were assessed in female Cyclops bicuspidatus thomasi experimentally infected with procercoids of Triaenophorus crassus. Copepods were given daily rations of Paramecium caudatum, from zero per day to ad libitum. Host fecundity and procercoid growth were significantly reduced at lower rates of host feeding but no direct effects of parasite presence on host fecundity were detected. Procercoid growth was nutrient limited but some procercoid growth occurred in copepods with no food intake. More procercoids were differentiated within 10 days in hosts fed four or more Paramecium per day than in hosts fed fewer. The results indicate that a knowledge of host food intake is required for evaluation of the inter-relationships of larval cestodes with their crustacean intermediate hosts.

INTRODUCTION

One of the final events in the annual phase of the life cycle of Triaenophorus crassus is the ingestion of coracidia by cyclopid copepods and their subsequent development into the procercoid larva. In Manitoba waters the copepod most commonly infected is Cyclops bicuspidatus thomasi (Watson and Price 1960; Watson and Lawler 1965). Several aspects of the Cyclops-Triaenophorus relationship have been examined previously (Miller 1943b; Rosen and Dick 1983; Shostak et al. 1984, 1985) and indicate the presence of intensity-dependent procercoid growth and parasite-induced copepod mortality. Competition for nutrients between host and parasite or between parasites is possibly the underlying factor responsible for those observations, but has not been tested experimentally.

Studies on a variety of copepod-cestode systems suggest that nutritional factors are frequently involved. Reduced fecundity of infected hosts is reported from natural (Evans 1983) and experimental (Kuperman and Kireev 1976) infections. Infection with larval cestodes often leads to reduced host activity (Herde 1938; Miller 1943b; Mueller 1959) and presumably to a decrease in host feeding rate as well. Host feeding rate influences host fecundity (Lanciani 1975; Patrick 1984) and size of larval cestodes (Mueller 1959). Observations of reduced fecundity in infected crustaceans could result

from competition between host and parasite for nutrients, but could also result from indirect effects of the parasite on host feeding behavior. The nutritional relationships between larval cestodes and their crustacean intermediate hosts are therefore unclear.

The life history of C. b. thomasi is similar to that of other cyclopid copepods (Patrick 1984). This species is multivoltine, and passes through six naupliar, five copepodid, and one adult stage. Following mating, eggs pass from the uterus to a pair of external brood sacs, where each clutch (= one pair of egg sacs) is carried until the eggs hatch. Several clutches are produced, in succession, from a single mating. Cyclops b. thomasi is carnivorous (Stemberger 1985) and will cannibalize (Patrick 1984; T.A. Dick, personal communication). The objective of this study was to use experimental infections and controlled feeding of C. b. thomasi to examine the relationships between copepod feeding rate, copepod fecundity, and growth and development of procercoids of T. crassus.

MATERIALS AND METHODS

DATA COLLECTION

Four experiments were undertaken in 1984 to assess various aspects of the relationship of host food intake to parasite growth and host fecundity (Table 31). Copepods were collected from the Fort Whyte Nature Center in Winnipeg, Manitoba. This source of copepods is free of natural infections with T. crassus based on annual spring spot checks for procercoids from 1979-1984. The copepods were maintained as stock cultures in a refrigerator at 8C, and were used 1.5-3 wk after collection. Food was not added to the stock culture, and predation or cannibalism within the stock culture was not prevented. Eight hours prior to each experiment, adult female C. b. thomasi which possessed eggs within the uterus and/or within egg sacs were selected and placed individually in 1.5 ml of dechlorinated water without food. Eggs from ca. 20 T. crassus were pooled and maintained in dechlorinated water at 15C on a 12 h light: 12 h dark photoperiod and monitored daily for hatching of coracidia. All coracidia used in infections were less than 24 h old. Paramecium caudatum, maintained

Table 31. Daily rations of *Paramecium* provided to copepods (unexposed, and exposed to *T. crassus*).

Experiment	Copepod group	Feeding treatment per copepod group
A	Exposed	0(45)[a], 7(45)
	Unexposed (control)	0(12), 7(12)
B	Exposed	0(30), 2(29), 4(30), 6(28), 21-97[b](30)
C	Exposed	0(20), 5(40), 34-107[b](40)
	Unexposed (control)	0(17), 34-107[b](30)
D	Unexposed	0(6), 2(6), 4(6), 6(6), 10(6), 60-150[b](6)

[a] No. *Paramecium*·copepod^{-1}·day^{-1} (no. of copepods).

[b] *ad libitum* feeding.

according to the procedures of Rosen (1983) were the food source for controlled feeding of copepods. The same stock of coracidia, copepods, and Paramecium was used for all experiments. Within each experiment copepods were allocated at random to exposed or control groups, and to various feeding treatments. All experiments were set up at room temperature, but copepods were stored at 15C on a 12 h light: 12 h dark photoperiod for the remainder of the experiment.

A 10-day experimental period was chosen to maximize the time available for parasite growth while minimizing the number of infected copepods lost through mortality. Rosen and Dick (1983) showed that about 75% of the growth in linear dimensions of procercoids is completed within 10 days, and that peak host mortality occurs 13-16 days after infection.

To evaluate infections, copepods were killed in 70% ethanol, stained with aqueous acetocarmine, dehydrated in ethanol, cleared in methyl salicylate, and mounted in synthetic resin.

Protocol for experiment A.— Copepods were allocated to control (unexposed) or exposed groups, with two feeding treatments per group (Table 31). On day 0 copepods were exposed to an average of 102 coracidia each. These coracidia remained until eaten by the copepods or until they died. Paramecium were added to the fed group daily, from 1-9 days postexposure (PE) to coracidia. On day 10 PE surviving copepods were counted, killed, and prepared as whole mounts.

Protocol for experiment B.— On day 0 all copepods were exposed to an average of 10 coracidia each for 22 h, after which water was

changed to remove uneaten coracidia. Copepods were then allocated to one of five feeding treatments (Table 31). Each copepod was observed daily from days 1-10 PE for (1) mortality, (2) presence or absence of uneaten Paramecium, (3) presence or absence of egg sacs or nauplii in the water. Water was then changed to remove uneaten Paramecium and nauplii, and the daily ration of Paramecium added. The experiment was terminated on day 10 PE and remaining copepods were killed and prepared as whole mounts.

Protocol for experiment C.— Copepods were allocated to control and exposed groups, with two feeding treatments in the control group and three feeding treatments in the exposed group (Table 31). On day 0 copepods were exposed to an average of 53 coracidia for 14 h, after which water was changed. Each copepod was observed daily from days 1-10 PE for (1) mortality, (2) presence or absence of uneaten Paramecium, (3) presence or absence of egg sacs or nauplii in the water, (4) presence or absence of egg sacs on the copepod. Water was then changed and the daily ration of Paramecium added. The experiment was terminated on day 10 PE and remaining copepods were killed and prepared as whole mounts.

Protocol for experiment D.— Copepods, with egg sacs, were allocated to one of six feeding treatments (Table 31). Daily observations for 10 consecutive days were made on each copepod to count the total number of eggs in each pair of egg sacs and determine the time of formation and release of each clutch. Water was then changed and the daily ration of Paramecium added.

DATA ANALYSIS

Data are presented as $\bar{X} \pm SD$. All volumes are in nanolitres (nl). Statistical procedures follow Sokal and Rohlf (1981). Statistical significance was determined using $\alpha = 0.05$.

Host mortality and size, infection prevalence and intensity (Expts. A-C).— Host mortality was evaluated as the number of copepods dead on day 10 PE, relative to the initial number of live copepods. Mortalities in exposed and unexposed groups were compared using the G-test. Host size was determined using volume estimates from whole-mounted copepods, assuming an ellipsoid shape for the cephalothorax and a half-ellipsoid for the abdomen. Host sizes were compared using a one-way analysis of variance (ANOVA). Prevalence was the number of infected copepods out of the total number exposed. Intensity was the number of procercoids in each infected copepod.

Confirmation of host feeding (Expts. A-D).— Natural mortality of Paramecium was assessed in a separate experiment in which seven groups of 70 Paramecium each were placed in 1.5 ml of water with no copepods present, and stored under the conditions of experiments A-D. The number remaining after 24 h was used to evaluate survival, and was the basis for evaluating natural death of Paramecium when copepods were present.

Host fecundity (Expts. B-D).— Fecundity was assessed by daily observations of clutch production and release, since the large number of copepods observed daily did not permit exact counts of egg numbers

to be made on all individuals. Copepods were assumed to have released one clutch of eggs (Expt. B) if egg sacs or nauplii were seen in the water during the daily observations. The number of clutches released per copepod during the 10-day experimental period was compared between feeding treatments using an RxC test of independence with the G-statistic. To estimate the number of clutches produced (Expt. C) the total number of days in which females were observed carrying egg sacs was divided by the median observed time between clutch formation and release. The number of clutches produced per copepod in the exposed group was compared between feeding treatments using an ANOVA with orthogonal comparisons. Student's t-test was used to compare clutch production between the unexposed and exposed groups. The time from clutch formation to release was compared between control and infected groups, and between feeding treatments, using the G-test. The number of eggs per clutch (Expt. D) was compared between feeding treatments using ANOVA. Data from the egg sacs carried by the copepods at the start of experiment D were not used.

Parasite growth and differentiation (Expts. A-C).— The volume of each procercoid was estimated assuming an ellipsoid shape. Parasite growth was assessed using total procercoid volume per copepod, since individual procercoid size is intensity-dependent. Data were grouped for analysis as follows: three feeding regimes (unfed; 2-7 *Paramecium* added daily; *ad libitum*) seven intensity groups (1, 2, 3, 4-8, 9-13, 14-18, 19-25 procercoids/copepod). Comparisons of total procercoid volume (following logarithmic transformation) were made between feeding treatments at corresponding intensities, using Student's t or ANOVA with multiple comparisons between all pairs of means using least

significant differences. Procercoids were classed as differentiated if they possessed a cercomer or frontal invagination. All others were classed as undifferentiated. Comparisons between feeding treatments were made using an RxC test of independence with the G- statistic; data were pooled (0-2 *Paramecium* daily; 4 *Paramecium* to *ad libitum* daily) to maintain expected frequencies > 5 per cell.

RESULTS

Host mortality and size, infection prevalence and intensity (Expts. A-C) (Table 32).— Mortality data from the two feeding regimes in experiment A were pooled, and no difference found between control and exposed copepods ($G= 0.303$; $df= 1$; $P= 0.580$). In experiments B and C mortality was less than 2% in exposed and control groups, and was not compared statistically. The volumes of infected copepods were similar between experiments ($F= 2.11$; $df= 2, 280$; $P= 0.121$). Prevalence and intensity were high in experiment A. Prevalence and intensity were low, and similar, in experiments B and C.

Confirmation of host feeding (Expts. A-D).— In the absence of copepods the number of *Paramecium* present after 24 h was 1.051 ± 0.085 times the number introduced. No *Paramecium* remained after 24 h in 1363 of 1364 observations on copepods fed 10 or fewer *Paramecium*. One or more *Paramecium* remained after 24 h in 1282 of 1438 observations on copepods fed 21-150 *Paramecium*.

Host fecundity (Expts. B-D) (Table 33).— Copepods released 0, 1, or 2 clutches (Expt. B). The number of clutches released did not vary among copepods fed known numbers of *Paramecium* (0, 2, 4, or 6 per day) ($G= 5.25$; $df=3$; $P= 0.154$), but when those data were pooled, the number of clutches released by copepods fed 0-6 *Paramecium* per day was lower than for copepods fed *ad libitum* ($G= 21.5$; $df=1$; $P< 0.001$).

Table 32. Experimental infection of female C. b. thomasi with procercoids of T. crassus 10 days post-exposure to coracidia.

Experiment	Copepod group	Host		Parasite	
		Mortality	Volume (nl)	Prevalence	Intensity
A	Exposed	12.2 (90)[a]	0.32 ± 8.2 (52)[b]	100 (52)[a]	11.4 ± 5.7 (52)[b]
	Unexposed (control)	8.3 (24)	-	-	-
B	Exposed	2.1 (142)	0.34 ± 8.7 (135)	51.1 (135)	2.2 ± 1.3 (69)
C	Exposed	1.0 (100)	0.35 ± 6.4 (96)	59.4 (96)	2.3 ± 1.3 (57)
	Unexposed (control)	0 (47)	-	-	-

[a] Percent (no. of copepods).

[b] $\bar{X}$ ± SD (no. of copepods).

Table 33. Fecundity of female C. b. thomasi relative to food intake and infection with procercoids of T. crassus over a 10-day period. One clutch = one pair of egg sacs.

No. Paramecium added per day	Exposed copepods		Unexposed copepods
	No. clutches released (Expt. B)	No. clutches produced (Expt. C)	No. clutches produced (Expt. C)
0	0.58 ± 0.50 (26)[a]	0.64 ± 0.34 (18)	0.60 ± 0.31 (17)
2	0.74 ± 0.45 (27)	-	-
4	0.47 ± 0.51 (30)	-	-
5	-	0.96 ± 0.50 (40)	-
6	0.68 ± 0.48 (28)	-	-
Ad libitum	1.03 ± 0.57 (29)	1.72 ± 0.32 (40)	1.77 ± 0.44 (30)

[a] $\bar{X}$ ± SD (no. of copepods).

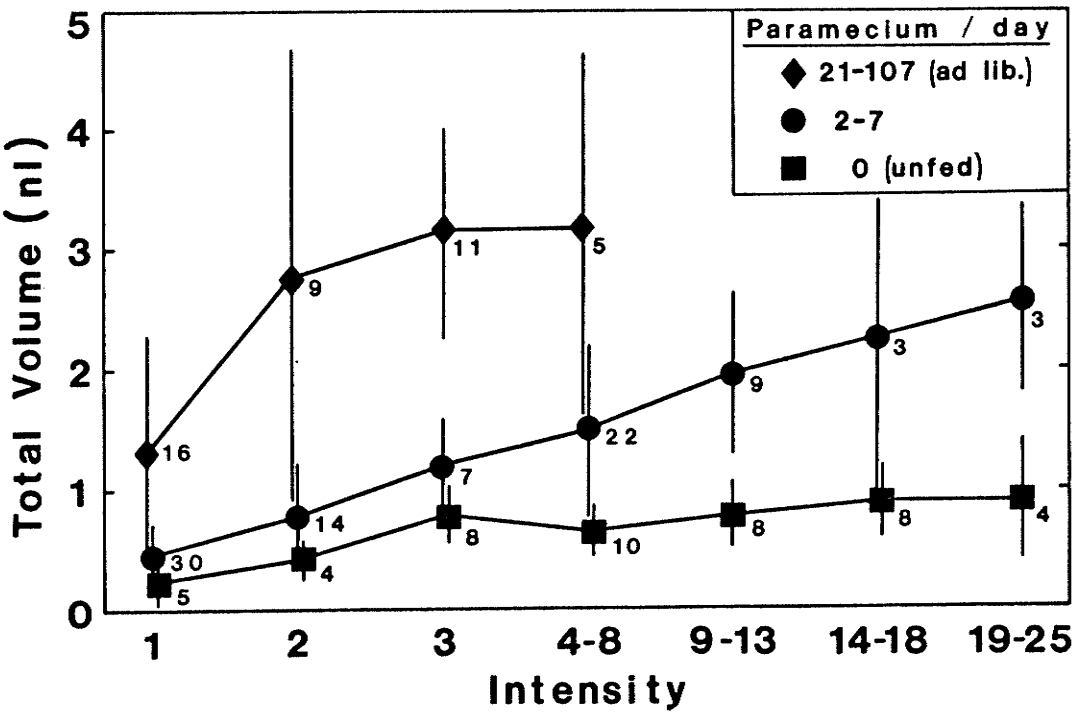
The time between clutch formation and release was determined from observations on 211 clutches in experiments C and D. Two clutches (1%) were released on the third day, 129 (61%) on the fourth day, and 80 (38%) on the fifth. The median time was four days in exposed and unexposed groups in all feeding treatments. The two egg sacs on each copepod were occasionally released on consecutive days, but usually on the same day.

In experiment C egg sacs were observed on copepods for 0-9 days of the 10-day experimental period, and since a given pair of egg sacs was carried for a median time of four days, females were calculated to have produced 0-2.25 clutches (no. days with egg sacs/ median time from egg sac formation to release). The number of clutches produced did not differ between the unfed-control and unfed-exposed groups ($t= 0.197$; $df=17$; $P= 0.423$) or between the control and exposed groups fed ad libitum ($t= 0.455$; $df=33$; $P= 0.326$). Among the exposed copepods those fed ad libitum produced more clutches than those fed at lower levels ($F= 106.5$; $df= 1, 95$; $P< 0.001$), and copepods fed 5 Paramecium daily produced more clutches than unfed copepods ($F= 7.97$; $df= 1, 95$; $P= 0.006$).

Each clutch contained 24 ± 6.7 eggs ($N= 28$) (Expt. D) and this number was similar among feeding treatments over the experimental period ($F= 1.99$; $df= 5, 22$; $P= 0.119$).

Parasite growth and differentiation (Expts. A-C) (Fig. 26).— Data on total procercoid volume per copepod were available from experiments B and C for intensities of 1 and 2; from experiments A, B, and C for intensities of 3 and 4-8; from experiment A for intensities of 9-13,

Figure 26. Changes in total volume of procercoids of T. crassus per female C. b. thomasi with intensity of infection and host food intake. All infections are 10 days old. Data are pooled over experiments A-C and are presented as $\bar{X} \pm SD$ with numbers indicating sample size of copepods.


Paramecium / day
21-107 (ad lib.)
2-7
0 (unfed)
Total Volume (nl)
0
1
2
3
4
5
1
2
3
4-8
9-13
14-18
19-25
Intensity
16
9
11
5
30
14
7
22
9
3
3
5
4
8
10
8
8
4

14-18, and 19-25. No significant differences in total procercoid volumes were detected between experiments at comparable intensities and feeding treatments, and since the copepods were of similar size the results of the three experiments were pooled for further analysis (Fig. 26). Total procercoid volume per unfed copepod initially increased, but at three or more procercoids levelled off at 2.8 $\pm$ 1.1% of host volume (N= 38 copepods). Total procercoid volume in copepods fed 2-7 Paramecium daily continued to increase with intensity, and reached 7.0 $\pm$ 3.0% of host volume (N= 3 copepods) at intensities of 19-25 procercoids. Total procercoid volume initially increased with intensity in copepods fed ad libitum, but levelled off at 8.8 $\pm$ 3.0% of host volume (N= 16 copepods) at intensities of two or more procercoids. Pairwise comparisons indicated that total procercoid volume differed significantly between feeding treatments ($P< 0.05$), with only one exception (intensity= 2 procercoids; unfed vs. 2-7 Paramecium daily).

Procercoids < 0.2 nl were not differentiated at day 10 PE at any feeding level. Only procercoids > 0.2 nl were evaluated for differentiation. In copepods fed 0-2 Paramecium daily, 0 of 63 procercoids were differentiated; in copepods fed 4 Paramecium to ad libitum daily, 31 of 257 procercoids were differentiated ($G= 12.9$; $df= 1$; $P< 0.001$).

DISCUSSION

This is the first study to control and quantify food intake by a freshwater crustacean infected with a larval cestode. The ad libitum feeding regime was confirmed by the presence of uneaten food the following day, and the ingestion of all food when copepods were given 10 or fewer Paramecium was confirmed indirectly as none remained the following day. Furthermore, controls showed that numbers of Paramecium did not decline in the absence of copepods. It was not determined whether infected copepods increased their food intake to compensate for nutrients aquired by growing procercoids. Compensatory feeding was possible in the ad libitum feeding regime since there was an excess of food, but not on feeding regimes of 10 or fewer Paramecium per day. Nauplii were not periodically removed in experiment A and were likely cannibalized when present (Mueller 1966), but probably did not contribute significantly to energy intake by the copepods, as procercoid sizes in experiment A did not differ at comparable intensities from procercoid sizes in experiments B and C (where nauplii were removed by daily water changes).

Copepod fecundity was proportional to food intake by uninfected and infected copepods. The low frequency of clutch production in unfed copepods, and the 2-3-fold increase in copepods fed ad libitum in this study, supports observations of Peacock (1982). Peacock

(1982) also reported that the number of eggs per clutch for C. b. thomasi was about 20% lower in habitats with reduced food availability, but actual food intake was not quantified. By contrast, in this study the number of eggs per clutch was independent of host food intake, and the timing of clutch release was constant. Consequently, clutch production was considered to be a direct measure of fecundity over the course of these experiments. Restrictions in host fecundity were not directly attributable to the presence of the parasite in this study. This was surprising, as it has been suggested (Lanciani 1975; Kuperman and Kireev 1976; Keymer 1980; Evans 1983) that parasites reduce fecundity in crustacean and insect hosts. Perhaps (1) procercoids of T. crassus were poor competitors for host nutrients, or utilized nutrients not required by the host for egg production, or (2) the low intensities ($\bar{X}$= 2.3 procercoids) and short duration of experiment C produced an insufficient energy drain on the host to reduce host fecundity. Host fecundity was not assessed from high intensity infections in this study, but the results of experiments on the closely-related Triaenophorus nodulosus suggest that a reduction in fecundity would be minimal. They found that Cyclops strenuus infected with 15-18 procercoids produced only 20% fewer eggs than did uninfected females.

Availability of nutrients has been suggested to limit growth or development of larval cestodes in invertebrates (Michajlow 1953; Voge 1959; Guttowa 1961; Rosen and Dick 1983) but food intake by the hosts was not quantified. In this study food intake by the parasite was indirectly manipulated by varying food availability to the host. The addition of known numbers of Paramecium and confirmation of their

ingestion established host food intake ranging from no dietary input to ad libitum feeding. Host fecundity was proportional to food intake, indicating that the amount of food ingested appeared to be directly correlated with nutrient concentrations in the hemolymph. In unfed hosts procercoid volume was limited to 2.8% of host volume. That limit could be determined by (1) space availability, in which case procercoid size should remain proportional to host size with increased nutrient availability, or (2) nutrient availability, in which case procercoid size relative to host size would increase. It was demonstrated that increasing host food intake increased absolute size of procercoids, and relative volume of parasite to host, which reached 8.8% on an ad libitum feeding regime. Thus it has been experimentally established that nutrient availability is a limiting factor on procercoid growth. Nutrient limitations may be the factor responsible for intensity-dependence or procercoid size. It can be seen in Figure 26 that the size of individual procercoids declined with increasing intensity, since total procercoid size did not continue to increase with intensity. This crowding effect was also noted in previous studies of individual procercoid size in this system (Rosen and Dick 1983; Shostak et al. 1984, 1985).

Low intensities (1-3 parasites per host) are common for natural infections of small freshwater crustaceans with larval cestodes (Watson and Lawler 1965; Kuperman 1973; Evans 1983). These low intensities were represented in this study, and the range of host food intake in the experiments spanned the natural range of food intake by the copepods, at least in terms of quantity (no food to ad libitum feeding). The results suggest an important role for host food intake

on the host-parasite relationship under natural conditions. Parasite growth and developmental rates varied directly with host food intake, yet no direct reduction in host survival or fecundity as a result of parasitic infection could be demonstrated. Furthermore, many procercoids were able to complete development with minimal effects on the copepod during the first 10 days of infection.

The average maximum size per procercoid of T. crassus in infections with 1-5 procercoids is about 1 nl (extrapolated from Figs. 3 and 4 of Rosen and Dick [1983]) and is reached by day 17 PE. In the present study, over the same range of intensities, average size per procercoid was also about 1 nl, but was reached within 10 days in copepods fed ad libitum. This suggests that copepods in the study of Rosen and Dick (1983) were underfed, but also implies that maximum somatic size of the parasite can be reached sooner with increased intake of food by the host. Rosen and Dick (1983) suggested that the mortality of infected copepods at days 13-16 PE was due to mechanical or nutritional stress on the host. By contrast, comparably-sized procercoids to those observed by Rosen and Dick (1983) grew in hosts fed ad libitum in this study but the lack of host mortality indicates that damage due to the physical size of the parasite may not be the cause of host death. However, a higher proportion of the procercoids of the same size were differentiated in the study of Rosen and Dick (1983) than in this study, and differentiation of the procercoid has been suggested to involve an increase in its nutrient absorption capabilities (Kuperman 1973). Consequently, increased nutrient demands by differentiating and differentiated procercoids, in the presence of lower host food intake, may be sufficient to cause host

death through nutritional stress. This interpretation may explain the differences in host mortality between this study and the study of Rosen and Dick (1983). In the present study total procercoid volume was manipulated by controlling host feeding rate, and the generation of varied infection intensities in conjunction with control of host feeding may be used to manipulate the size of individual procercoids. The ability to experimentally manipulate parasite size and the rate of differentiation through the intake of food by the host appears to be a useful tool for further studies on causes of parasite-induced host mortality.

Two important points emerge from this study. First, variations in host food intake are expected to occur in natural systems (Watson and Price 1960), and it was demonstrated that host food intake has a significant effect on parasite growth and development at infection intensities frequently encountered in natural systems. Second, the observation that host fecundity and parasite growth were directly related to host food intake indicated that both were nutrient limited. But additive effects of limited nutrients and the parasite on host fecundity were not detected even when food intake by the host was controlled. Consequently, before one attributes direct effects of a parasite on a host in natural or experimental systems, the energy requirements of the host and the host-parasite complex must be considered.

CHAPTER 6: TRIAENOPHORUS CRASSUS IN THE FIRST INTERMEDIATE HOST

ABSTRACT

Eggs were collected separately from 18 Triaenophorus crassus obtained from spawning northern pike at Falcon L., Manitoba in 1983 and 20 T. crassus in 1984. Eggs were maintained in dechlorinated water at 15C on a 12 h light: 12 h dark photoperiod. Coracidia from each lineage (= offspring of the same adult worm), collected within 24 h of hatching, were exposed to individual Cyclops bicuspidatus thomasi at a concentration of 100 coracidia/copepod. Exposed copepods were maintained under the same temperature and light conditions, and fed five Paramecium caudatum daily, for 10 days, after which infections in female copepods were evaluated. Coracidia hatched over a period of five days within a lineage, but the day of hatch did not affect resulting infection intensities. The volume of individual procercoids, at intensities of 1-5 procercoids/copepod, varied significantly between copepods exposed to coracidia of the same lineage, but did not vary between lineages. Prevalence and intensity of infection, procercoid volume, and copepod survival, varied between years while an index of procercoid differentiation did not. The cause of this annual variability was not conclusively established. The host fish that a lineage originated from had a minor effect on most aspects of the infection, but variability among individual copepods was a major environmental source of phenotypic variability in parasite characteristics.

INTRODUCTION

Many cestodes with aquatic life cycles produce a free-swimming coracidium, containing an oncosphere, that is ingested by a copepod first intermediate host. The oncosphere penetrates through the gut wall into the hemocoele of the copepod and develops into a procercoid larva that is able to infect a second intermediate host fish. The mechanisms by which copepods and coracidia contact each other under natural conditions are unknown, but events following infection are reasonably well documented by extensive experimental studies, primarily dealing with species of Triaenophorus, Diphyllobothrium, and Schistocephalus.

Following penetration into the hemocoele the oncosphere grows, and at 1-2 wk begins differentiation to the procercoid by forming a cercomer and frontal invagination (Miller 1943b; Clarke 1954; Guttowa 1961; Halvorsen 1966; Kuperman 1973; Rosen and Dick 1983; Shostak et al. 1985). For convenience all stages within the copepod will be referred to as procercoids. A minimum age of ca. eight days and a minimum volume of ca. 0.2 nl are required before differentiation occurs (Shostak et al. 1985). Following differentiation growth continues for a short period, then ceases (Miller 1943b; Clarke 1954; Kuperman 1973; Rosen and Dick 1983; Shostak et al. 1985). Differentiation is required for infectivity to the second intermediate

host (T.A. Dick, personal communication). The procercoid may live as long as the copepod (Miller 1943b, 1952; Kuperman 1973), but high infection intensities increase the probability of copepod mortality (Rosen and Dick 1983). A "crowding effect" (Read 1951) occurs in all species examined (Miller 1943b; Michajlow 1953; Clarke 1954; Guttowa 1961; Halvorsen 1966; Kuperman 1973; Rosen and Dick 1983; Shostak et al. 1984, 1985), and was shown for Triaenophorus crassus to be due to nutrient limitations (chapter 5).

Considering the importance of biological variability for the evolution of a species, relatively little is known concerning variability in characteristics of the procercoid-copepod relationship. A few things are known about variability in procercoid growth (Michajlow 1953; Guttowa 1961; Halvorsen 1966; Shostak et al. 1984, 1985), but a number of significant questions have not been addressed. These questions include: (1) Do coracidia differ in ability to infect, grow, and differentiate in copepods? (2) How does variability within a sex or age class of the copepod host affect the parasite? (3) Are some procercoids in a population more pathogenic to the copepod than others? Previous studies do not provide sufficient information about the organisms or protocols used to answer these questions.

In my study, adult female copepods Cyclops bicuspidatus thomasi were exposed to coracidia of T. crassus, and subsequently maintained, under controlled conditions. The histories of coracidia were known from the adult worms that produced them, and copepods used within a year came from a common source. The objectives of this study were to evaluate characteristics of coracidia from different adult worms with

respect to: (1) infectivity to the copepod, (2) growth and differentiation of procercoids within the copepod, and (3) pathogenicity to the copepod.

MATERIALS AND METHODS

CORACIDIA

Eggs were collected from individually-identified adult T. crassus during 1983 (18 worms from 7 northern pike) and 1984 (20 worms from 6 pike) according to the methods in chapter 3. The eggs, coracidia, and procercoids from the same adult worm will be termed a lineage.

Each lineage was maintained in 200 ml erlenmyer flasks containing 175 ml dechlorinated water and 250,000 eggs at 15C on a 12 h light: 12 h dark photoperiod. Each flask was examined daily for the presence of coracidia in the water column. Coracidia normally aggregated in a "cloud" near the top of the flask. An estimate of numbers of coracidia was made by removing them from the flask with a pipette and placing the suspension in a 125x15 mm test tube. After mixing gently by aspirating with a pipette each of 10 drops of the suspension was placed on a glass spot plate and the coracidia counted. When there were sufficient numbers of coracidia in a flask they were used to infect copepods. Usually 5-20 drops of suspension were required to provide an inoculation dose of 100 coracidia/copepod. A check on

accuracy of this procedure used 10 drops from a common source replicated five times, and resulted in 102 $\pm$ 8.2 coracidia/10-drop sample. Therefore, ca. 95% of samples ($\pm$ 2 SD) were within 16% of the mean.

A preliminary experiment using coracidia pooled from all lineages found that 50% of coracidia lived up to 36 h, but none lived more than three days, in the experimental conditions noted above.

A sample of coracidia was pooled from all lineages in 1984 to examine the range of variability in oncosphere volumes within the parasite population. These were fixed and stained using procedures followed for infected copepods (chapter 5) except they were cleared in xylene. The coracidia were mounted en masse and the first 200 observed during scanning a slide were selected to measure length and width of the enclosed oncosphere.

Coracidia within a lineage hatched over a period of one to several days. In 1983 freshly-hatched coracidia were selected on the first and second day of hatch (DOH) for exposure to 30 randomly-selected copepods. In 1984 a morning and afternoon infection using 20 randomly-selected copepods for each was done on DOH 1 and DOH 2 for all lineages. To test for the effect of DOH on the infectivity of coracidia, five lineages from three pike were chosen in 1984, and morning and afternoon infections with freshly-hatched coracidia were done on 20 randomly-selected copepods from the first to the last DOH.

COPEPODS

Copepods were collected approximately one week prior to their anticipated use each year from waters on the eastern shore of Lake I, a 6.4 ha, man-made lake in the Fort Whyte Nature Center, Winnipeg, Manitoba (Loadman 1980). Each year about 1200 C. b. thomasi (ca. 70% adult females, 25% adult males, and 5% fifth stage copepodites) were used as a common source that copepods were selected from at random for exposure to coracidia as previously described. Copepods were exposed and maintained individually in 75x10 mm numbered glass test tubes containing dechlorinated water, at 15C on a 12 h light: 12 h dark photoperiod. Water levels in test tubes were maintained at 2.5-3.0 ml by the addition of dechlorinated water when required (every 2-3 days). Copepods were fed an average of five Paramecium caudatum/ day (see methods in chapter 5 for details) but were not fed on the day of exposure to coracidia.

On the 10th day postexposure (PE) to coracidia surviving copepods (adult females and males, copepodites) were counted, and all survivors killed and prepared as whole mounts as described in chapter 5. Length and width of all procercoids was measured using an occular micrometer, and procercoids were classed as differentiated if they had a cercomer and/or a frontal invagination, or undifferentiated if they had neither.

DATA ANALYSIS

Information on the past history of each lineage (section of intestinal attachment of the adult worm, host fish, year of collection) was recorded as described in chapter 3.

Oncosphere volumes were estimated from length and width measurements by assuming they had an ellipsoid shape. Mean, variance, and coefficient of variation (CV= $100 \times SD/\bar{X}$) were calculated from measurements of the pooled source.

Prevalence and intensity were determined using female copepods living on day 10 PE. A single prevalence (proportion of copepods with at least one procercoid) was calculated for each lineage. Intensity (number of procercoids per infected copepod) was calculated for each copepod, and a mean intensity was calculated for each lineage.

Procercoid volumes were estimated from length and width measurements by assuming them to have an ellipsoid shape, using infections in female copepods. A mean, variance, and CV were calculated for individual procercoid volumes within a copepod. Since procercoid size is intensity-dependent and various intensities of infection were obtained, analysis of covariance (ANCOVA) was used to estimate mean procercoid size for a lineage, adjusted for the effects of different intensities within a lineage.

Procercoids $\geq$ 0.2 nl volume in female *C. b. thomasi* have a high probability of differentiating (Shostak et al. 1985). Therefore I determined the proportion of procercoids $\geq$ 0.2 nl in female copepods

that were differentiated on day 10 PE in female copepods as an index of inter-lineage differences in procercoid development. I assumed that most procercoids $\geq$ 0.2 nl would eventually differentiate and that differences observed at day 10 PE indicated different rates of differentiation.

Parasite pathogenicity was assessed by copepod survival following standard exposure to coracidia. Copepods dead on day 10 PE were often degenerated and could not be separated by sex or stage. Therefore, copepod survival estimates for a parasite lineage were based on total numbers alive on day 10 PE (adult females and males, copepodites) relative to total numbers at the start of the experiment. It was assumed that the random allocation procedure did not bias the proportions of female, male, and copepodites exposed to each lineage.

Nested analysis of variance (ANOVA) was used to evaluate inter-lineage sources of variability for all variables (prevalence, intensity, procercoid volume, proportion of differentiated procercoids, and copepod survival). Nested ANOVA was used to assess intra-lineage variation in intensity due to the time of day (AM or PM) or DOH that coracidia were used for infections. Nested ANOVA was also used to evaluate intra-lineage variability in individual procercoid volumes at intensity= 1 (representing the usual intensity observed in natural infections) and at each of intensities= 2-5 (to permit separation of inter-procercoid variability within a copepod from the effects of inter-copepod variability in procercoid volumes).

Nested ANOVAs were done using SAS while ANCOVA was done using programs in the APL public library of the University of Manitoba

Computer Services. Statistical significance was determined using α=0.05.

VOUCHER SPECIMENS

A slide containing whole-mounts of three copepods with 10-day-old procercoids of *Triaenophorus crassus* is deposited in the National Museum of Natural Science, Ottawa, Canada K1A 0M8, NMNS catalogue number NMCP1986-0839.

RESULTS

ONCOSPHERE VOLUME

The frequency distribution of oncosphere volumes was slightly skewed (Fig. 27). Mean volume was 0.0124 ± 0.0038 nl (N= 200) and the coefficient of variation (CV) was 30%.

PREVALENCE

Between 20% and 100% of female copepods exposed to 100 coracidia of various lineages aquired infections (Fig. 28A). Lineages originating from different host fish varied in prevalence, and prevalence varied significantly between years (Table 34). A lower proportion of copepods was infected from the 1983 lineages (Fig. 28A). Only ca. 12% of variance in prevalence among lineages was not accounted for by section of intestinal attachment, host fish, or year of collection.

Figure 27. Frequency distribution of oncospheres measured within coracidia of T. crassus. UCL, upper class limit.

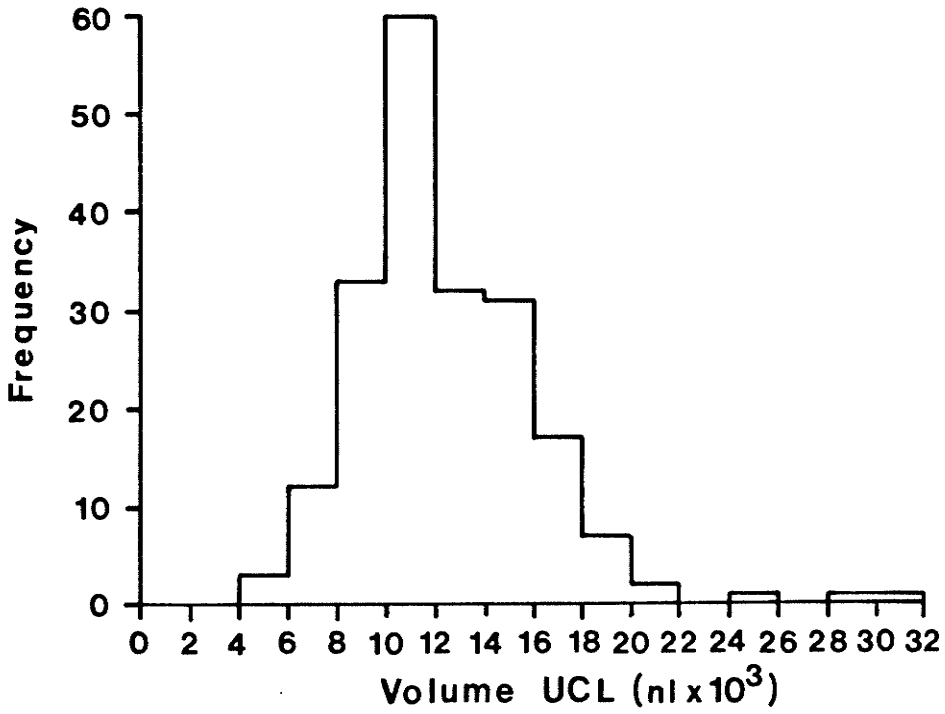

60
50
40
30
20
10
0
Frequency
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Volume UCL (nl x 10^3)

Figure 28. Frequency distributions of prevalence and intensity of infection of female C. b. thomasi by different lineages of T. crassus, determined 10 days postexposure to coracidia. A. Percentage of copepods infected with at least one procercoid of T. crassus. B. Mean number of procercoids per infected copepod.

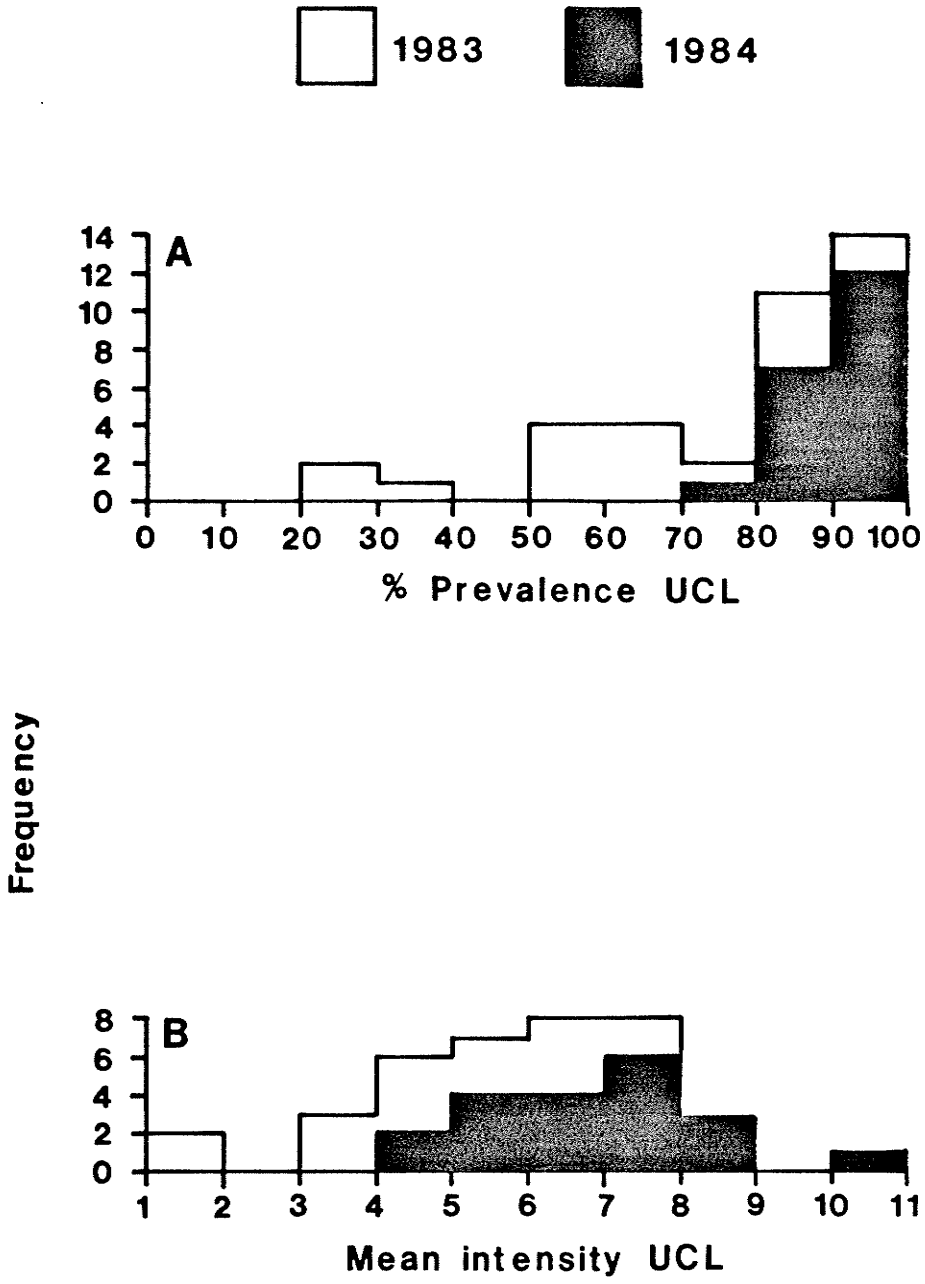

1983
1984
A
14
12
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100
% Prevalence UCL
Frequency
B
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11
Mean intensity UCL

Table 34. Nested analysis of variance on the proportion of copepods infected with procercoids of T. crassus. Data were transformed by arcsin $\sqrt{P}$.

Source	df	MS	% Variance component	Significance test[a]
Year	1	1.17222	45.13	F'= 5.381; df= 1, 10; P= 0.041
Fish	11	0.13790	40.87	F'= 8.220; df= 11, 8; P= 0.004
Section	10	0.01654	1.51	F= 1.186; df= 10, 15; P= 0.371
Error	15	0.01394	12.49	
Total	37	0.08280	100.00	

[a] F' is approximate test using Satterthwaite approximation.

INTENSITY

As part of a preliminary experiment five lineages from three fish in 1984 were used to assess changes in infectivity of coracidia relative to DOH (Fig. 29). Sufficient numbers of coracidia to conduct exposures were present for 3-5 days. Since coracidia hatching on a given day were removed for infections, coracidia present the following day were all freshly hatched. A consistent pattern of intensity relative to DOH was not observed (Fig. 29). The data from these five lineages and other lineages in 1984 (that did not span the entire egg hatching period) were segregated by DOH and time of day (AM or PM) that coracidia were removed for exposure to copepods (Table 35). Over 93% of variance in infection intensities in 1984 could not be explained by lineage or by the time and DOH that coracidia were removed for exposure (Table 35). It was concluded that coracidia hatching at different times were similar and all data from infections by a lineage in female copepods were used in subsequent analyses.

The mean intensity of infection varied between lineages, from 1-11 procercoids/copepod (Fig. 28B). Nested ANOVA revealed marginally-significant ($P < 0.10$) added variance in mean intensity among lineages due to host fish and year of collection (Table 36), and intensities were slightly lower in 1983 (Fig. 28B) although ranges overlapped broadly between years.

Figure 29. Mean numbers of procercoids of T. crassus in female C. b. thomasi 10 days postexposure to coracidia that hatched on different days within a lineage. Points represent $\bar{X} \pm$ 95% confidence limits. Uninfected copepods were included in calculation of the mean. Each graph is from a different lineage of T. crassus.

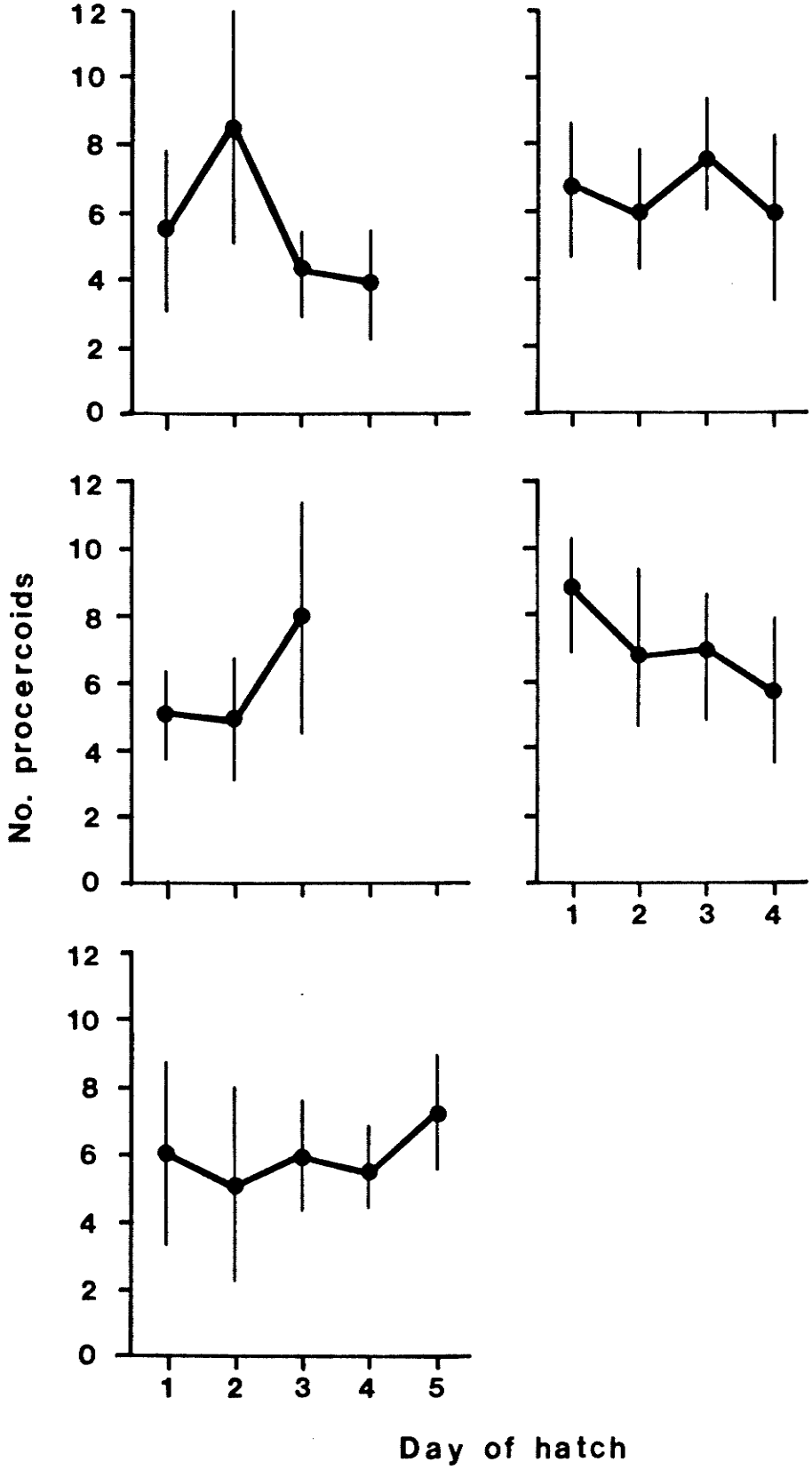

12
10
8
6
4
2
0
No. procercoids
1
2
3
4
5
Day of hatch

Table 35. Nested analysis of variance on intensity of T. crassus in experimentally-infected copepods (1984 data only). Data were transformed by ln(x+1).

Source	df	MS	% Variance component	Significance test[a]
Lineage	19	0.76922	0.00	_[b]
Day of hatch	23	1.18914	5.91	F'= 1.904; df= 23, 14; P= 0.107
Time of day	17	0.62077	0.75	F= 1.077; df= 17, 563; P= 0.373
Error	563	0.57643	93.35	
Total	622	0.60619	100.00	

[a] F' is approximate test using Satterthwaite approximation.

[b] Violates conditions for Satterthwaite approximation.

Table 36. Nested analysis of variance on mean intensity of T. crassus in experimentally-infected copepods. Data were transformed by ln(x+1).

Source	df	MS	% Variance component	Significance test[a]
Year	1	0.84663	27.99	F'= 4.624; df= 1, 7; P= 0.067
Fish	11	0.13238	24.63	F'= 2.623; df= 11, 8; P= 0.091
Section	10	0.05121	0.00	F= 0.863; df= 10, 15; P= 0.583
Error	15	0.05932	47.38	
Total	37	0.10013	100.00	

[a] F' is approximate test using Satterthwaite approximation.

PROCERCOID VOLUME

The volume of individual procercoids varied with intensity but also among procercoids within a copepod. This variation was evaluated for infections with a single procercoid, and for infections with 2-5 procercoids (low to moderate levels of crowding). In single procercoid infections there was a significant effect of year (Table 37). Procercoids in the 1983 collection were generally larger, and there was an effect of section of attachment of the adult worm. Most variation in procercoid volume within a year occurred among procercoids of the same lineage, as indicated by the large error variance (Table 37) but in single infections it could not be determined whether this comprised intrinsic variation in volume of procercoids or effects of their occurrence in different host individuals. An analysis of copepods with multiple infections (Table 37) indicated differences in volume among years of collection, but showed that a significant proportion of within-lineage variance in volume could be accounted for by variation between copepod hosts. Interestingly, at intensity= 2, the error variance component was only 15% but was larger at higher intensities. Differences between lineages accounted for small, non-significant added variance in procercoid volume in multiple infections (Table 37).

The CV for volume per procercoid within a copepod was 45-76%, but did not vary with either intensity (over the range 4-11 procercoids/copepod) or size of the breeding population of T. crassus (1-29 sexually-mature worms). One lineage used in this study was from

Table 37. Summary of percent variance components from nested analyses of variance on individual procercoid volumes of T. crassus in C. b. thomasi at five intensities. Data were transformed by ln(nl).

Source	Intensity				
	1	2	3	4	5
Year	13*	30**	28**	25**	26***
Fish	0^{ns}	0^{ns}	0^{ns}	1^{ns}	0^{ns}
Section	25*	25*	0^{ns}	12^{ns}	7^{ns}
Lineage	0^{ns}	0^{ns}	9^{ns}	0^{ns}	2^{ns}
Copepod	} 62	30***	16**	18***	28***
Error		15	46	44	37
Total	100	100	100	100	100
No. procercoids	57	118	195	292	375

Note: ns, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

a lone sexually-mature T. crassus that probably self-fertilized; CVs at three intensities were 45-60%.

In each lineage the volume of individual procercoids varied within and between copepods at each intensity, and varied inversely with intensity. The standard copepod exposure protocol for each lineage produced a range of intensities. ANCOVA was used to incorporate procercoid volume information from all intensities and adjust for the effect of intensity on volume. To provide independent measurements for the ANCOVA a mean procercoid volume was calculated for each copepod; measurements of individual procercoids were not independent since the presence of other procercoids in a copepod could affect growth. Plots of mean volumes vs. intensities showed a curvilinear relationship. Transformation of data to ln(volume) and $\sqrt{\text{intensity}}$ produced plots that seemed linear for most lineages, but this was difficult to assess due to much scatter. ANCOVA indicated no difference in slopes among lineages ($F= 1.238$; $df= 37, 671$; $P= 0.160$) but differences in intercepts ($F= 7.344$; $df= 37, 608$; $P< 0.001$). However, there was significant ($P< 0.001$) heterogeneity in the data at all levels of the analysis and therefore results of the ANCOVA were accepted with caution. Adjustment of data for intensity used the common slope ($b= -0.770$) fitted through the mean (X, Y) of the transformed data for each lineage, and the volume of a procercoid predicted from this line at intensity= 1 was chosen as a representative size for each lineage. To confirm that adjusted procercoid volumes accurately reflected observed differences among lineages, I assessed at each intensity the correlation between observed volume of a lineage and the adjusted volume (Table 38).

Table 38. Correlations between mean observed procercoid volumes (where means are based on $N \geq 2$ copepods) and adjusted volumes based on analysis of covariance.

Intensity	N[a]	r	Significance test
1	12	0.81	t= 4.367; df= 10; P< 0.001
2	16	0.83	t= 5.568; df= 14; P< 0.001
3	14	0.81	t= 4.785; df= 12; P< 0.001
4	18	0.75	t= 4.536; df= 16; P< 0.001
5	16	0.90	t= 7.726; df= 14; P< 0.001
6	16	0.76	t= 4.375; df= 14; P< 0.001
7	15	0.87	t= 6.362; df= 13; P< 0.001
8	15	0.72	t= 3.741; df= 13; P= 0.001
9	12	0.74	t= 3.479; df= 10; P= 0.002
10	10	0.65	t= 2.419; df= 8; P= 0.021
11	9	0.93	t= 6.694; df= 7; P< 0.001
12	4	0.96	t= 4.849; df= 2; P= 0.043
13	3	0.67	t= 0.903; df= 1; P= 0.266
14	5	0.87	t= 3.056; df= 3; P= 0.027

[a] Number of lineages with copepods at specified intensity.

Ideally, a perfect correlation (r= 1) should result. Calculated correlation coefficients were high and positive (Table 38). Moreover, when data at each intensity were further segregated by year, there were still positive correlations between adjusted and observed volumes in 15 of 18 comparisons. Therefore I considered the adjusted volumes for each lineage to be acceptable representations of relative procercoid growth to day 10 PE among the lineages examined. Adjusted procercoid volumes varied among lineages from 0.18-1.37 nl (Fig. 30A). There was a significant difference between lineages among years, but not due to host fish or section of attachment by the adult worm (Table 39). Adjusted procercoid volumes were larger in 1983, although there was overlap in values from the two years (Fig. 30A).

PROCERCOID DIFFERENTIATION

The proportion of differentiated procercoids $\geq$ 0.2 nl on day 10 PE varied between lineages, from 0-80% (Fig. 30B). The bimodality evident in Fig. 30B reflected a slight tendency of lineages originating from the same host fish to have a similar proportion of differentiated procercoids, but this effect was only marginally-significant (Table 40). The proportion of differentiated procercoids did not vary between years (Table 40).

COPEPOD SURVIVAL

Figure 30. Frequency distributions for procercoid volume and proportion of differentiated procercoids of T. crassus in female C. b. thomasi 10 days postexposure to coracidia. A. Procercoid volume in each lineage adjusted to represent intensity= 1 procercoid/copepod. B. Proportion of procercoids > 0.2 nl that were differentiated.

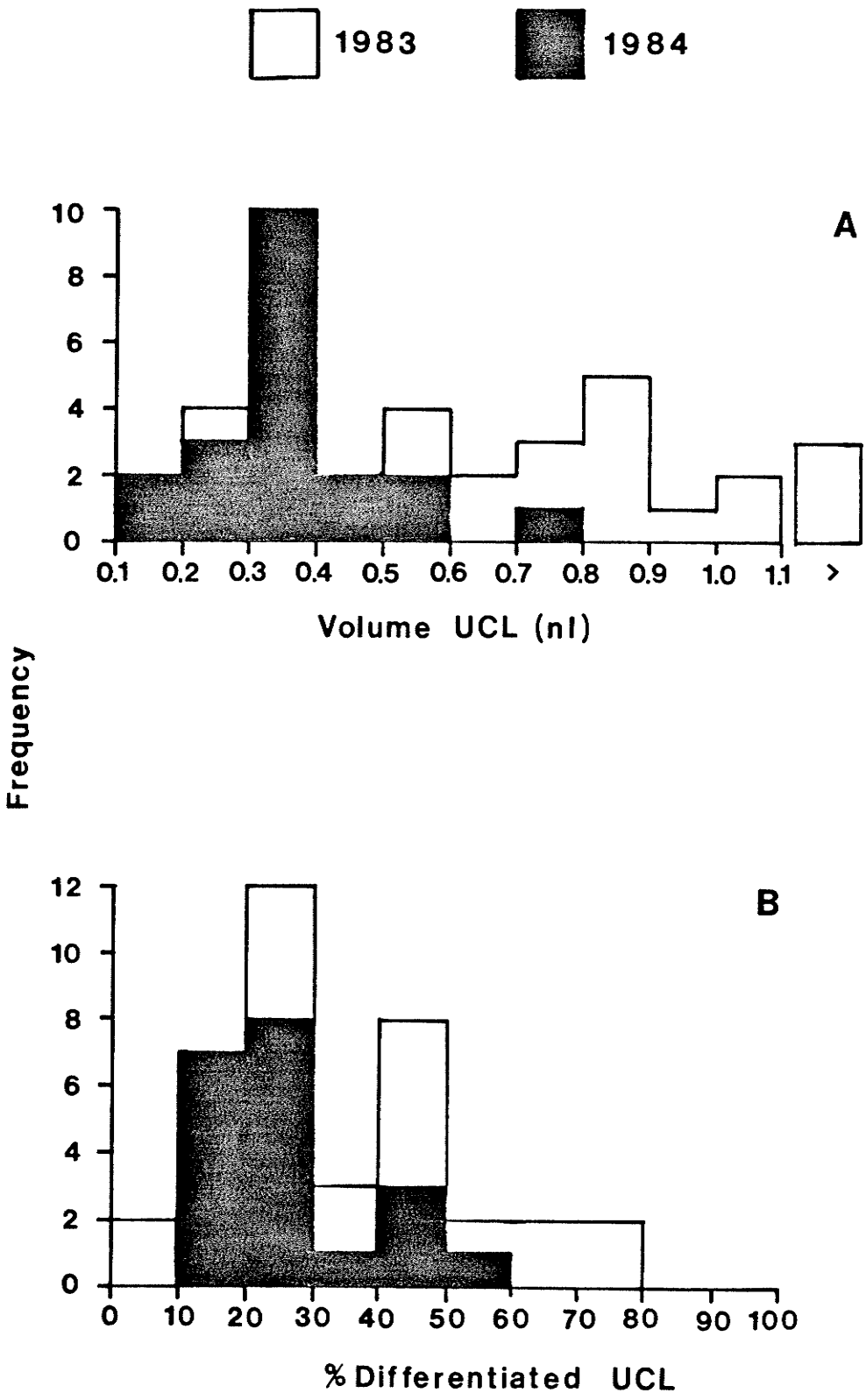

1983
1984
A
10
8
6
4
2
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
>
Volume UCL (nl)
Frequency
B
12
10
8
6
4
2
0
0
10
20
30
40
50
60
70
80
90
100
% Differentiated UCL

Table 39. Nested analysis of variance on adjusted procercoid volume of T. crassus. Data were transformed by ln(n1).

Source	df	MS	% Variance component	Significance test[a]
Year	1	6.65863	71.78	F'= 37.49; df= 1, 4; P= 0.005
Fish	11	0.15675	1.66	F'= 1.154; df= 11, 8; P= 0.432
Section	10	0.13391	2.90	F= 1.188; df= 10, 15; P= 0.370
Error	15	0.11273	23.66	
Total	37	0.30846	100.00	

[a] F' is approximate test using Satterthwaite approximation.

Table 40. Nested analysis of variance on the proportion of procercoids larger than 0.2 nl that were differentiated. Data were transformed by arcsin $\sqrt{P}$.

Source	df	MS	% Variance component	Significance test[a]
Year	1	0.16955	1.02	F'= 1.072; df= 1, 8; P= 0.332
Fish	11	0.10845	43.36	F'= 2.761; df= 11, 8; P= 0.080
Section	10	0.03806	14.31	F= 1.532; df= 10, 15; P= 0.221
Error	15	0.02485	41.31	
Total	37	0.05719	100.00	

[a] F' is approximate test using Satterthwaite approximation.

Survival of copepods to day 10 PE was generally high, ranging from 63-97% (Fig. 31). Survival of copepods differed between years (Table 41) and was greater in 1983 (Fig. 31). Copepod survival did not vary with respect to the section of attachment or host fish harboring the adult worm of an infecting lineage (Table 41).

Figure 31. Frequency distribution of percentage of C. b. thomasi surviving for 10 days after exposure to 100 coracidia from different lineages of T. crassus. Copepods were a mixture of adult females and males, and fifth stage copepodites.

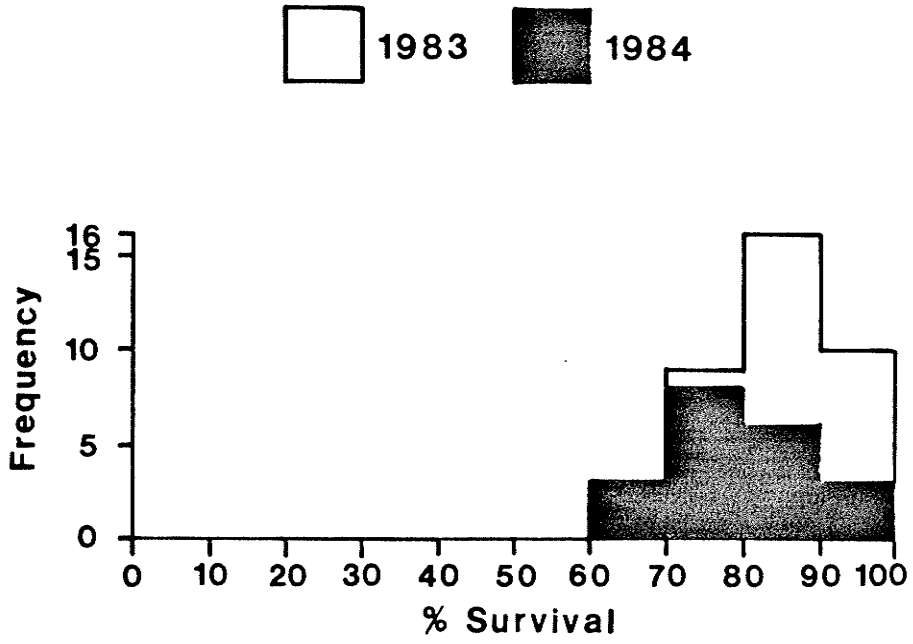

1983
1984
Frequency
16
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100
% Survival

Table 41. Nested analysis of variance on the proportion of copepods exposed to T. crassus that survived for 10 days. Data were transformed by arcsin $\sqrt{P}$.

Source	df	MS	% Variance component	Significance test[a]
Year	1	0.14085	36.95	F'= 10.62; df= 1, 4; P= 0.032
Fish	11	0.01245	0.00	F'= 0.956; df= 11, 8; P= 0.540
Section	10	0.01270	12.42	F= 1.376; df= 10, 15; P= 0.281
Error	15	0.00923	50.63	
Total	37	0.01468	100.00	

[a] F' is approximate test using Satterthwaite approximation.

DISCUSSION

The primary purpose of this study was to evaluate the effects of the past history of a coracidium on its infection of the copepod, relative to the effects of the biotic environment provided by the copepod. Past history included not only genetic inheritance from adult T. crassus but also environmental effects on phenotype accumulated between egg formation and hatching of a coracidium. Previous studies on larval cestodes in invertebrate hosts did not address the role of past history and consequently there was no prior information on what characteristics to use in selecting parasite lineages. To increase the likelihood that lineages chosen would represent diverse past histories a large number of adults were selected. The characteristics chosen (prevalence and intensity, growth and differentiation of procercoids, and host survival) reflected the collective effects of numerous interactions between copepod and parasite. Nevertheless, it was possible to identify effects of past history of a coracidium and current environment (copepod) on the process of infection by coracidia, and growth and differentiation of procercoids, of T. crassus. Prior to discussing the effects of past history and current environment substantial differences between years noted for several characteristics must be addressed.

Variations between years were expressed as lower intensities and

prevalences for 1983 than for 1984, but larger adjusted procercoid volumes and host survival for 1983. Perhaps these differences were due to differences in experimental conditions or intrinsic differences in the parasites or copepods used. Variations in experimental conditions were probably minor as physical conditions during maintenance of copepods and parasites were controlled within narrow limits, and infection protocols and feeding routines were standardized. Variations in number of inoculated coracidia around a mean of 100 was low, and that number was well in excess of the ability of copepods to handle prey of that size. Cyclops b. thomasi rarely consumed more than 21 Paramecium/day (chapter 5) and rarely ingest more than 30 coracidia (Miller 1943b), while the larger Mesocyclops edax ingests no more than 40 prey/day (Williamson 1984). Variations in food rations between years was probably not a factor since the feeding experiments conducted in 1984 (chapter 5) showed that ad libitum feeding ($\geq$ 21 Paramecium/day) would be necessary to produce procercoids in 1984 as large as those in 1983 yet rations in both years were five Paramecium/day. Consequently the most likely explanation is that there were intrinsic inter-year differences in coracidia or copepods. It was noted in chapter 4 that hatchability of eggs was about 10% lower in 1983 than 1984, while median egg hatch times were longer by about one day. This may indicate a small difference in viability of coracidia between years, but does not explain the larger procercoid volumes in 1983 unless there was a tradeoff between ability of a coracidia to infect a copepod and its ability to subsequently grow. Differences between years were more likely attributable to differences between the two samples of

copepods. Although copepods were collected from the same site on Lake I in both years, Patrick (1984) found annual variations in biological characteristics of C. b. thomasi within a population. The composition of the copepod samples (proportions of males, females with and without egg sacs, and 5th stage copepodites) was similar but the background of each sample was unknown and may have involved differences in age, nutritional status (chapter 4) or innate characteristics of physiology, resistance, or feeding behavior.

Three sources of parasite biological variability in the T. crassus -copepod relationship could be evaluated with the data from this study. The first source was past history in terms of the environment of adult T. crassus from which lineages originated. Each lineage was characterized by mean intensity, adjusted procercoid volume, etc., and evaluated in a three-level environmental hierarchy of year, host fish within year, and section of attachment by the adult within the intestine of the host fish. There was a pronounced year effect but by controlling for this it was found that host fish accounted for some differences between lineages but section of attachment did not. Variation among lineages originating from adult worms attached in the same intestinal section was as low as 12%; this included effects of measurement error, unidentified environmental effects, and genetic differences among lineages. Past history summarized by this three-level environmental hierarchy explained much of the variation between lineages.

The second source of variability was past history in terms of phenotypic variability in hatch times of eggs within a lineage. It

was found in chapter 4 that host fish had a strong influence on the length of time required for eggs from a lineage to hatch. If late hatching eggs have reduced infectivity, as suggested by Guttowa (1955) in a study on T. nodulosus, transmission dynamics of the parasite might be altered. I measured infectivity of coracidia throughout the hatching period under standardized exposure conditions, in contrast to the study of Guttowa (1955), and found that the time of day or day of hatch had a negligible effect on infection intensity. Most variability was among copepods exposed at the same time to the same source of coracidia. This indicated that late-hatching eggs were not less viable, and also that the feeding behavior or physiology of the individual copepod, not past history of the coracidium, had a major influence on the infection process.

The third source of variability was the copepod host and its relationship to individual procercoid growth as assessed by volume. It was important to evaluate this source since procercoid volumes in single infections were highly variable within a lineage; single infections are the usual case in natural infections (Watson and Lawler 1965; Esch 1983), and procercoid size is suggested to have consequences for transmission (Clarke 1954; Shostak et al. 1984, 1985). I determined for copepods with multiple infections that a significant amount of variation in procercoid volume was due to the copepod host, although there was still intra-copepod variability.

There are a number of reasons for intra-copepod variability of procercoid volumes given that all procercoids are of the same lineage. (1) Genetic diversity. This was not examined directly, but one

lineage was from a single gravid worm recovered from a pike, presumably self-fertilized with more homozygous offspring than worms from larger breeding infrapopulations, but intra-copepod variability was similar to variability in presumably outcrossed lineages.

(2) Phenotypic variability in eggs of a lineage. Egg volumes were variable, but the coefficient of variation (CV) within a lineage was small (8-14%, chapter 4). Oncospheres within coracidia were more variable in volume, and had a larger CV (ca. 30%). The CV for individual procercoid volumes within a copepod was 45-76%. This trend of increasing variability in size suggests that existing phenotypic variability at one stage was amplified in the next.

(3) Differences in procercoid age. Procercoids are growing rapidly by day 10 PE (Shostak et al. 1985), and since coracidia lived up to three days under experimental conditions, procercoids could have been as young as seven days old on day 10 PE. However, most coracidia were probably eaten on the first day, and therefore procercoids were probably 9-10 days old on day 10 PE. The magnitude of this age difference can be assessed using growth curve equations for procercoids of T. crassus (Shostak et al. 1985). For example, assuming that four coracidia are eaten at random times within the first 24 h after inoculation and using the appropriate growth formula (Shostak et al. 1985), expected procercoid volumes on day 10 PE would have a CV of 7-16% (based on 10 randomizations). This variability is clearly less than the minimum CV of 45% observed for infection with four procercoids, and therefore variations in infection age only partially contributed to intra-copepod procercoid volume variability.

(4) Intraspecific competition. In a unimodal population, suppression

of growth in smaller individuals by larger ones can lead to size bimodality (Huston 1986) and consequently increased size variability. This situation likely exists for procercoids of T. crassus since larger procercoids can differentiate sooner and increase their growth rate at the expense of smaller procercoids which remain stunted (Shostak et al. 1985). This interpretation is consistent with the observation of higher error (intra-copepod) variability in infections with three or more copepods than when only two procercoids were present.

The unique contributions of these four factors to phenotypic variability in procercoid volume cannot be conclusively determined, particularly for genetic effects, but it seems that much of the variability was due to environmental effects such as age differences, competition, and prior phenotypic variability. The increasing variability from egg to oncosphere to procercoid suggests that a small amount of initial variability, possibly with a large genetic component, is probably amplified and clearly confounded by subsequent environmental effects. By the procercoid stage variability in biological characteristics can no longer be traced to genetic differences. The resolution of this question may require that individual offspring be characterized through the entire sequence from egg formation to hatching of coracidia to infection of the copepod.

Two events following infection of the copepod must occur to make successful transmission possible. The procercoid must differentiate and the copepod must survive so differentiated procercoids are available to the second intermediate host. The time for initiation of

differentiation varies from 8 to 16 or more days PE (Kuperman 1973; Rosen and Dick 1983; Shostak et al. 1985). In my study 10 days PE was used and therefore it was not possible to determine if lineages differed in the number of procercoids that would eventually differentiate, only that the time when differentiation occurred was variable. Interestingly, differentiation was the only characteristic evaluated in this study that did not vary between years, indicating that while host environment had a pronounced effect on procercoid acquisition and growth it did not influence this critical developmental process.

The lower survival of copepods in 1984 was clearly related to the higher prevalence and intensity of infection since procercoids can cause host mortality (Rosen and Dick 1983). However, survival to the 10th day was high and the data provided no indication that parasite lineages differed in pathogenicity to that point.

The most thoroughly-studied aspect of the T. crassus -copepod relationship was procercoid growth, since procercoid growth characteristics may influence the probability of differentiating (Shostak et al. 1985), copepod survival (Rosen and Dick 1983; Shostak et al. 1985), and chances for successful infection and growth in the second intermediate host (Clarke 1954; Rosen and Dick 1983; Shostak et al. 1984). Procercoid growth was assessed using volume estimates on day 10 PE, a time when the growth phase is close to completion (Rosen and Dick 1983; Shostak et al. 1985) and individual variability had been expressed. Several observations indicated that phenotypic variability in procercoid growth resulted not only from prior

variability in oncosphere size but also from plasticity on the part of individual procercoids to alter growth in response to immediate environmental (host copepod) conditions: (1) Intensity-dependent procercoid growth was observed in all lineages. Although not likely to be common in natural infections, this illustrated the flexibility in growth of procercoids originating from a common genetic pool when exposed to varying levels of relative nutrient availability. (2) Variability in procercoid growth within a lineage could be directly attributed to variation among copepods. (3) Innate differences in procercoid growth among lineages, i.e. those that could not be attributed to environmental effects, were minimal. I suggest that the ability of individual procercoids to modify growth in response to immediate host-environmental conditions is adaptive for a parasite that cannot predict conditions within the individual copepod it will infect, since these conditions vary with sex, stage, or nutritional status of the copepod (Clarke 1954; Rosen and Dick 1983; Shostak et al. 1985; chapter 5) and copepods exhibited little selectivity in the coracidia they ingested. It is interesting to speculate that this plasticity in individual procercoid growth, and low level of inter-lineage variability, is the result of selection for a common growth strategy by T. crassus that minimizes host stress to reduce (but not eliminate) parasite-induced host mortality. It is not known what proportion of the hundreds of thousands of coracidia produced by an adult T. crassus will successfully infect copepods, but it is probably low. There may be little latitude for evolutionary experiments within the procercoid stage.

CHAPTER 7: OVERVIEW OF THE ANNUAL PHASE OF THE LIFE CYCLE

ABSTRACT

Ten life-history characteristics were evaluated for 38 lineages of Triaenophorus crassus. Each lineage comprised (1) an adult worm from a naturally-infected northern pike Esox lucius from Falcon Lake, Manitoba, (2) its eggs and the coracidia that hatched from them under controlled conditions, and (3) procercoids resulting from experimental infections of the copepod Cyclops bicuspidatus thomasi to coracidia using a standardized protocol. Characteristics were adult mass and fecundity, proportion of eggs hatching, median time between egg release and hatching and its variance, prevalence and intensity of infection in the copepod, copepod mortality as a measure of parasite pathogenicity, and growth and differentiation of procercoids. Factor analysis using lineages as replicate observations indicated at least three clearly-defined common factors, each correlated with life-history characteristics expressed at a similar point in the life cycle. Characteristics expressed at different points such as fecundity and procercoid growth were not correlated with the same common factor. Nested analyses of variance were done separately for each characteristic and used the following hierarchy: year of collection, host fish, and section of intestinal attachment by the adult worm from which the lineage originated. These analyses showed that each level of the hierarchy was a source of variability for a different group of characteristics. The characteristics grouped on

basis of common sources of variance were similar to the groups identified by the factor analysis. It is concluded that T. crassus has several independent, coadapted groups of life-history characteristics, each associated with a specific part of the annual phase of the life cycle.

INTRODUCTION

The cestode Triaenophorus crassus has a complex life cycle that involves passage through four environments, with a different morphological stage of the parasite associated with each environment: coracidium in the free-living environment, procercoid in the first-intermediate host (copepod) environment, plerocercoid in the second intermediate host (coregonid fish) environment, and adult worm in the definitive host (northern pike) environment. The plerocercoid may live for three or more years (Miller 1952; Rosen and Dick 1984) and acts as a resting phase in the life cycle. The remainder of the life cycle I have termed the annual phase.

Transmission of T. crassus within this annual phase is facilitated by synchronization of predictable annual patterns of spatial distribution and abundance of the various hosts, and the presence of stages of the parasite infective to those hosts (Miller 1952; Lawler 1969). In previous chapters it was noted that, superimposed on the long-term predictability of the abiotic and biotic environments of T. crassus, there were many potential sources of short-term unpredictability. Correlated with this environmental unpredictability was a high degree of phenotypic variability in characteristics of the adult (chapter 3), egg and coracidium (chapter 4), and procercoid (chapter 6) of T. crassus. The relationships of over a dozen

characteristics of T. crassus to sources of variability in the abiotic and biotic environments were analyzed in those chapters, but each was treated in isolation. The relationship between characteristics was not evaluated.

The objective of this chapter was to (1) determine whether there was an underlying common relationship between the phenotypic expression of life-history characteristics within a lineage of T. crassus (an adult and its offspring), and (2) evaluate the relationship between phenotypic variability of T. crassus and the three environments it occupies during the annual phase of the life cycle.

MATERIALS AND METHODS

DATA COLLECTION

Eggs were collected separately from 18 T. crassus in 1983 and from 20 in 1984. Eggs were maintained under controlled conditions for monitoring of egg hatching characteristics and for experimental infection of copepods as described in chapters 3, 4, and 6. Each adult worm and its offspring were termed a lineage. The 38 lineages chosen were a sample from several hundred lineages evaluated for egg-hatching characteristics, and had large numbers of coracidia hatching for use in copepod infections. Lineages were selected such that at least six host fish were represented each year, and that some fish with few T. crassus and some with many were included.

Ten life-history characteristics were assessed on each lineage:

(1) adult mass (measured following drying at 70C for 48 h; chapter 3),

(2) fecundity (total number of eggs released by an adult worm after 20 h in dechlorinated water at 8C; chapter 3),

(3) proportion of eggs hatching (determined graphically from data

collected in the later stages of the egg hatching period; chapter 4),

(4) median egg hatch time (estimated median time between release of eggs by an adult worm and hatching of eggs, determined using probit analysis; chapter 4),

(5) variance of egg hatch time (variation in egg hatch time around the median, determined using probit analysis; chapter 4),

(6) prevalence in copepods (proportion of female copepods, exposed to coracidia from a lineage under standard conditions, in which at least one procercoid was found on day 10 postexposure (PE) to coracidia; chapter 6),

(7) intensity in copepods (mean number of procercoids in infected female copepods on day 10 PE; chapter 6),

(8) copepod survival (proportion of all copepods exposed to coracidia from a lineage under standardized conditions that were alive on day 10 PE; chapter 6),

(9) procercoid volume (average procercoid volume for a lineage, adjusted for variation due to intensity using analysis of covariance to represent volume when intensity= 1 procercoid/copepod; chapter 6), and

(10) procercoid differentiation (proportion of procercoids larger than 0.2 nl that were differented on day 10 PE; chapter 6).

DATA ANALYSIS

The extent to which the 38 lineages selected for this study were

representative of T. crassus in the sampled host population was assessed by (1) performing nested analysis of variance (ANOVA) on each characteristic within this restricted data set and comparing the results with nested ANOVAs based on the more extensive data sets used in chapters 3 and 4, and (2) comparing frequency histograms for each characteristic in the same way.

When a large number of potentially correlated variables is available for analysis, pairwise correlations are often not easily interpretable (Gorsuch 1983). A set of 10 variables such as the life-history characteristics of T. crassus involves 45 pairwise correlations. The technique of factor analysis evaluates relationships among large numbers of variables and expresses them in terms of a smaller number of terms, called common factors. Iterated principal factor analysis was done using SAS on the correlation matrix of variables with lineages as replicates, followed by varimax rotation. In previous chapters significant inter-year variability occurred that might cause spurious correlations when data from 1983 and 1984 were pooled. This was tested by calculating correlations between characteristics separately by year and comparing with correlations using pooled data; no major discrepancies in sign or magnitude of correlations was found. Prior to factor analysis all proportion measures were transformed by $\arcsin\sqrt{p}$, and mass, volume, and intensity data were transformed to logarithms. The median egg hatch time and variance of egg hatch time, both expressed in ln(days) following probit analysis, were used without further transformation. Scores for all lineages on the first three common factors were estimated using the regression method.

Variance components and coefficients of variation (CV= 100xSD/$\bar{X}$) determined previously for selected characteristics (chapters 3, 4, and 6) were examined here in summary to evaluate general patterns of change throughout the annual phase of the life cycle of T. crassus.

RESULTS

The lineages used were a biased sample from T. crassus in the host population in terms of mass and fecundity of adults (Fig. 17), although similar variance components were estimated in this sample in comparison to those based on more extensive data (Tables 20, 21); this bias resulted from the unsuitabilty of lineages producing few eggs and coracidia for exposures to copepods. Nevertheless, a wide range of adult mass and fecundity was represented in the lineages used in this chapter (Fig. 17). There was little bias in egg hatching characteristics since all but the most extreme values were included in the sample of 38 lineages (Figs. 23A,B, 24). Variance components of all hatching characteristics were also generally similar between this sample and the more extensive data set (Tables 27-29).

The data matrix used for the factor analysis is in Appendix II. A scree test (Cattell 1966) indicated the presence of four substantive common factors, and four factors were retained in the analysis. The result of factor analysis following orthogonal varimax rotation is shown in Table 42. An oblique promax rotation was also done to assess whether factors were correlated (Gorsuch 1983) but a similar factor structure to that shown in Table 42 and low (ca. 0.15) inter-factor correlations resulted. Therefore the more easily-interpretable orthogonal factors from the varimax rotation were used.

Table 42. Factor structure resulting from iterated principal factor analysis, with varimax rotation, on life-history characteristics of 38 lineages of T. crassus.

Characteristic	Factor				Communality
	1	2	3	4	
Procercoid prevalence	94[a]	-26	-2	-21	0.99
Procercoid intensity	64	33	20	34	0.67
Survival of copepods	-60	-4	3	-21	0.42
Fecundity	2	89	21	-5	0.85
Adult mass	6	60	-20	-4	0.41
Variance of egg hatch time	-9	51	36	-27	0.49
Procercoid size	-38	30	60	-12	0.62
Procercoids differentiated	20	-3	89	-8	0.85
Proportion of eggs hatching	15	-21	-17	70	0.59
Median egg hatch time	-22	-6	20	-12	0.12
Variance explained	1.946	1.750	1.488	0.823	6.01
Cumulative percent	19.5	37.0	51.9	60.0	

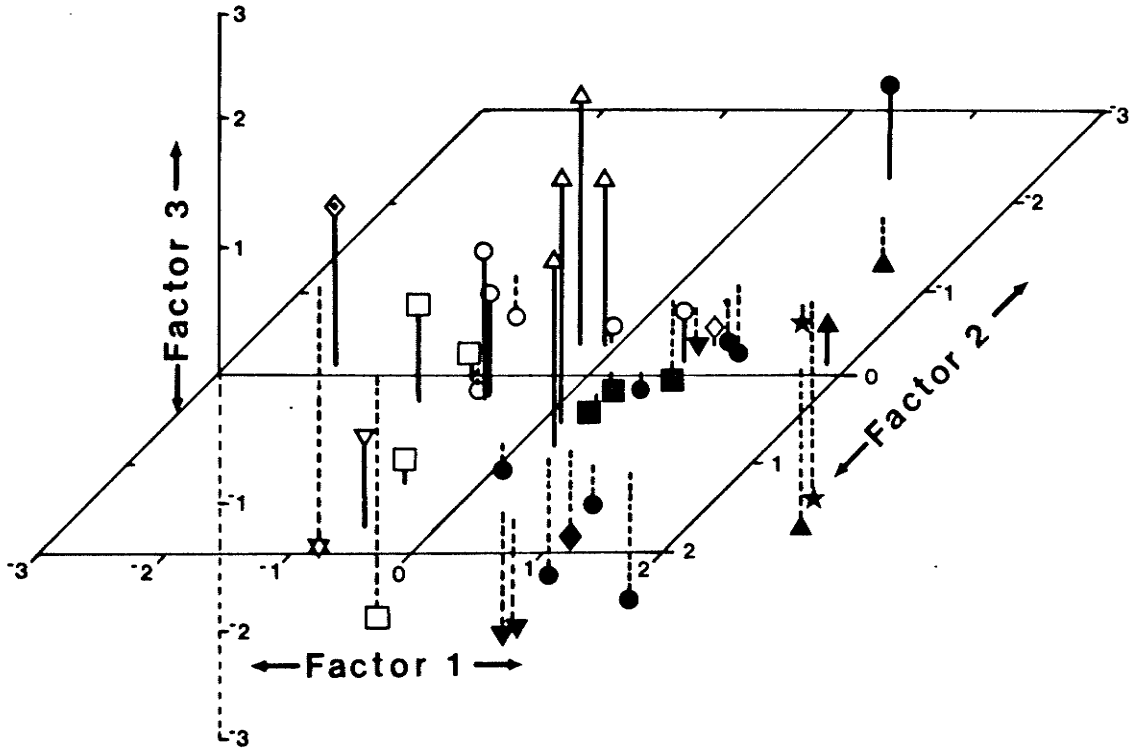
[a] Correlation of variable with factor, multiplied by 100 and rounded to the nearest integer. Underlined values indicate variables sharing at least 25% variance with that factor.

Factors are interpreted based on their correlations with variables, but small correlations may arise by chance. A minimum correlation of 0.50 was used for interpreting correlations of variables and factors using suggestions of Gorsuch (1983) and Bush and Holmes (1986a). Factor 1 correlated with variables measuring aspects of the infection process of copepods by the parasite: prevalence and intensity, and copepod survival (Table 42). Copepod survival was correlated with Factor 1 in an opposite manner to prevalence and intensity. Factor 2 was correlated with three variables measuring characteristics of the adult worm and its eggs: mass, fecundity, and variance of egg hatch time (Table 42). Factor 3 was correlated with procercoid growth and differentiation within the copepod (Table 42). The interpretation of Factor 4 was unclear since only one variable, the proportion of eggs hatching, was highly correlated with it (Table 42).

Most variables had moderate to high communality estimates, indicating that much of their variability could be explained by the common factors (Table 42). However, the majority of variance in median egg hatch time was unique to it; little was accounted for by the common factors (Table 42).

Estimated factor scores for all lineages on the first three factors were plotted (Fig. 32). There was clear separation among years on Factor 1 (aspects of copepod infections), but there was less separation among years on Factor 3 (procercoid growth), and almost none on Factor 2 (adult worm and eggs). Lineages originating from the same host fish had similar scores for Factor 1 and 3 but were less

Figure 32. Three-dimensional plot of scores for 38 lineages of T. crassus on Factors 1-3. Lineages originating from the same host northern pike are given the same symbol. Solid stems indicate positive values on Factor 3, dashed stems indicate negative values.


Factor 3
Factor 1
Factor 2
3
2
1
0
-1
-2
-3

similar for Factor 2 (Fig. 32).

Sources of variability for 10 life-history characteristics of T. crassus were evaluated in chapters 3, 4, and 6 using different data sets. To enhance interpretability of the variance components, only those calculated using the same set of 38 lineages are summarized in Table 43. The error component in these analyses included differences between lineages originating from adult worms attached within the same section of intestine of the host northern pike. The characteristics in Table 43 are listed in chronological order within the annual phase. Several trends are evident in the sizes of the variance components (Table 43): (1) The error variance (unexplained by section of attachment, host fish, or year of collection) was highest for adult mass and fecundity, and variance of egg hatch time (characteristics correlated with factor 2). (2) Section of attachment by the adult worm accounted for a moderate amount of variability in adult mass and fecundity, and in characteristics of the copepod infection, but not in egg hatching characteristics. (3) The individual host fish accounted for little variability in adult mass or fecundity, but a moderate to large proportion of variability in most other characteristics. (4) Inter-year variability was not observed in adult mass and fecundity, but was a moderate source of variability in egg hatching characteristics, and a prominent source for most aspects of the infection of copepods (Table 43).

Body size is an attribute common to all stages in the life cycle, and data were available to assess the relative magnitude of body size variation in T. crassus at all stages. Coefficients of variation were

Table 43. Variance components from analysis of variance on 10 life-history characteristics of T. crassus, using lineages for which data were available on all 10 characteristics.

Characteristics	Source			
	Error	Section	Fish	Year
Adult mass (type 3 worms)	79	15	6	0
Fecundity	61	39	0	0
Variance of egg hatch time	68	0	19	13
Median egg hatch time	27	0	73**	10
Proportion of eggs hatching	28	0	62**	10
Prevalence in copepods	12	2	41**	45*
Intensity in copepods	47	0	25†	28†
Survival of copepods	50	12	0	37*
Adjusted procercoid volume	24	3	2	72**
Proportion of procercoids differentiated	41	14	44†	1

Note: †, $p<0.10$; *, $P<0.05$; **, $P<0.01$.

calculated after pooling all available data for each stage to maximize sources of phenotypic variability (Table 44). Variation in egg volume had the lowest relative magnitude, and variability in size increased in successive life cycle stages, reaching a maximum in type 1 worms (defined in chapter 3). Variability then declined as worms matured (Table 44).

Table 44. Coefficients of variation (CV) for size of individual *T. crassus* in successive stages of the life cycle.

		N	$\bar{X}$	CV (%)
Egg volume		439	0.053 nl	19
Oncosphere volume		200	0.012 nl	30
Procercoid volume (intensity= 1)		57	0.59 nl	78
Plerocercoid mass[a]		53	2.4 mg	68
Adult mass:	type 1	818	5.5 mg	114
	type 2	75	19 mg	79
	type 3	188	38 mg	60

[a] from Southern Indian L. cisco.

DISCUSSION

A number of life-history characteristics of T. crassus were independently evaluated in previous chapters, and several sources of phenotypic variability in all characteristics were identified. There were two problems with interpreting the independent analyses. First, it was not possible to determine if these sources acted independently on each characteristic, or if two or more characteristics of a lineage responded as a unit to a common source. Second, environmental effects on phenotypic variability at different life cycle stages could not be directly compared because of the reduced and possibly biased data sets used for later life cycle stages. These problems were addressed in this chapter by (1) the use of factor analysis to help define relationships among characteristics, and (2) a re-examination of variance components to clarify relative effects of several sources of phenotypic variability.

An assumption of factor analysis is that there exist underlying hypothetical factors causally linked to two or more observable variables, and furthermore that each variable is also influenced by a factor unique to it (Gorsuch 1983). This assumption seemed reasonable in my study since the life-history characteristics evaluated were all related to aspects of parasite transmission and growth and might therefore have a common causal basis. There were few data in the

literature to formulate a priori expectations for the number of common factors. However, I thought one of two outcomes was likely. First, there may have been a single common factor correlated with all life-history characteristics, such as some attribute of an adult worm that would determine characteristics of its offspring throughout the remainder of the annual phase. Second, if life-history characteristics were compartmentalized, there may have been three common factors, one associated with each of the three environments of the parasite (definitive host, free-living, and copepod host) encountered during the annual phase. The number of variables and replicates used in the factor analysis was reasonable if up to three common factors were expected (Kim and Mueller 1978; Gorsuch 1983).

Factor analysis suggested that there was greater compartmentalization of life-history characteristics of T. crassus than I anticipated. There were (1) three common factors clearly correlated with characteristics expressed at specific points in the life cycle, (2) a fourth common factor that was less interpretable, and (3) a unique factor with a large effect on median egg hatch time. In view of this high degree of compartmentalization, the data base was not adequate to fully evaluate all underlying common factors; points 2 and 3 (above) require inclusion of additional variables and lineages for clarification (Gorsuch 1983). Nevertheless, the conclusion that life-history characteristics of T. crassus are compartmentalized is supported for three reasons: (1) Three factors had a clear biological basis. For example, Factor 1 involved the process of infection of copepods by coracidia, hence the relationship of prevalence and intensity or between them and copepod survival that is known to be

reduced by infection with T. crassus (Rosen and Dick 1983). (2) The life-history characteristics grouped together by factor analysis were also groups of characteristics that had similar sources of variability revealed by nested ANOVAs. (3) Characteristics strongly correlated with a given factor were ones expressed at a similar point in the life cycle, as has been found in studies of free-living invertebrates (Haukioja and Hakala 1978; Hegmann and Dingle 1982). By contrast, studies on fishes find correlations among characteristics expressed at different points in the life cycle (Trendall 1982; Able and Felley 1986).

Factor analysis was also useful in assessing the presence of tradeoffs among life-history characteristics. Tradeoffs are commonly found in interspecific comparisons (Hoagland and Schad 1978; Kuramoto 1978; Stearns 1980) but less so in intraspecific comparisons (Constantz 1979; Trendall 1982). A tradeoff between infectivity of coracidia and resulting copepod mortality was noted earlier, although this may be due to the high concentrations of coracidia used in experimental infections. The suggestion in chapter 6 that there was a tradeoff between infectivity and growth characteristics of coracidia and procercoids, respectively, was not supported since characteristics pertaining to infectivity and growth were correlated with different factors.

The relationship between host fish and several characteristics of larval T. crassus that was noted in chapters 4 and 6 was confirmed by the grouping of lineages by scores on Factor 1 and Factor 3. Furthermore, this indicated that lineages from the same host fish had

similar suites of characteristics, a conclusion that could not be made from the independent analyses of each characteristic done in previous chapters. The mechanism responsible for this host effect remains undetermined.

Each group of life-history characteristics of T. crassus was associated with a special part of the parasite's environment. Consequently, these characteristics probably represent several coadapted groups, each group evolving in response to selection pressures from one of the abiotic or biotic environments encountered by the parasite. Some of the possible selection pressures that may act independently in each environment were considered in previous chapters and included plasticity in growth (as shown by large coefficients of variation for parasite size) to adapt to individual variability of definitive and first intermediate hosts, and perhaps more importantly the need to synchronize dispersal characteristics of offspring with the host individual infected by the parent worm. One must be cautious in generalizing since these types of relationships have not been looked for in previous studies on helminths. However, Rose (1983) suggested, based on a survey of studies on free-living organisms, that evolution of groups of life-history characteristics is probably the usual case in natural populations.

GENERAL DISCUSSION

The life cycle of Triaenophorus crassus was treated as a series of separate components in most of the previous chapters but there were two recurrent themes that warrant some summary comments. These were (1) sources of phenotypic variability and (2) parasite site specificity within the definitive host.

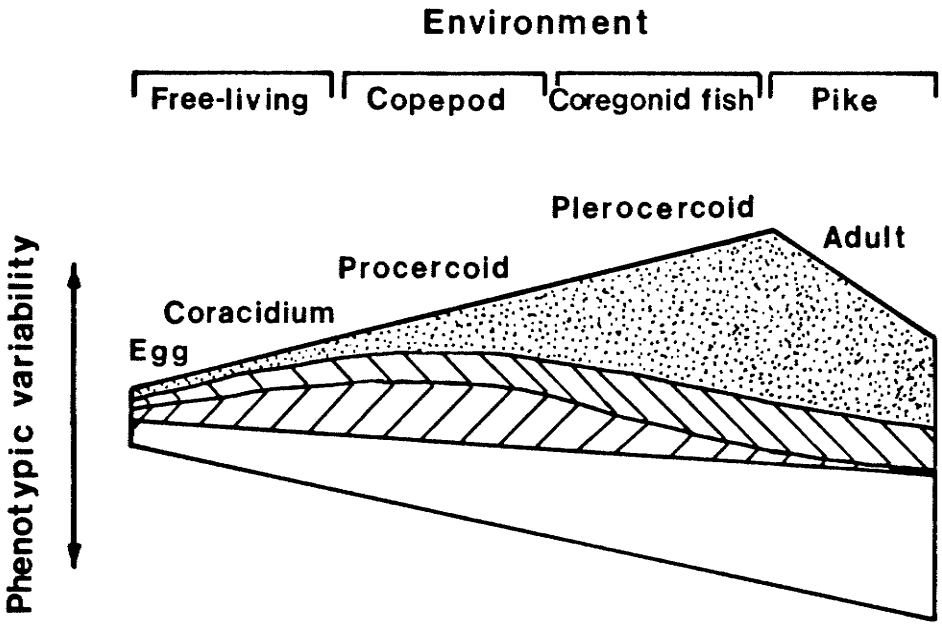
SOURCES OF PHENOTYPIC VARIABILITY

This study found a substantial amount of phenotypic variability in life-history characteristics, even when many variables, particularly those in the abiotic environment, were controlled. In previous chapters I speculated on relative contributions of environment and genotype to the observed phenotype using inferences made from the inability of various environmental variables (primarily the "black box" variables discussed in chapter 3) to explain observed phenotypic variability. However, this was done in the absence of genetic studies required to determine the exact nature and extent of genetic variability (Futuyama 1979). The genetics of life-history characteristics of T. crassus may remain a black box as its life cycle has yet to be completed in the laboratory and therefore controlled breeding studies are not feasible. However, estimates of environmentally-based variability in characteristics affecting fitness, from studies on Drosophila spp. and a variety of domestic animals, are about 50% (Futuyama 1979). It has also been found that genetic variability of these characteristics is due to coadapted gene

complexes as well as varying levels of additive genetic variability that is heritable (Futuyama 1979; Etges 1982; Rose 1983). In my study phenotypic variability of lineages was evaluated; each comprised the offspring of a single adult worm, although sperm may have been provided by an unknown number of other worms. Presumably offspring in a lineage were more genetically uniform than those from different lineages, and this provided some justification for discussing unexplained inter-lineage phenotypic variability in terms of genetic variability.

Figure 33 summarizes what I believe to be the relative magnitudes and sources of phenotypic variability in life-history characteristics of T. crassus, based on environmental effects examined in this study and a proportion of genetic effect that seems reasonable based on literature values for characteristics affecting fitness (Futuyama 1979). In general, phenotypic variability increased throughout the life cycle due to the effects of underlying genetic variability, variability caused by current environmental conditions, and environmental effects accumulated earlier in the life cycle (Fig. 33). Evidence for this increasing variability was found for body size measurements (chapter 7); the reduction in variability within the definitive host reflects the reduction in observed variability as worms matured in northern pike and may be due to a culling effect of the synchronized life cycle. Genetic variability is shown to represent about 30-50% of total variability (Fig. 33) since generally about 30-50% of variability was unexplained by environmental sources in previous chapters, and this range is similar to values for the genetic component of phenotypic variability in Futuyama (1979). The

Figure 33. Hypothetical changes in sources of phenotypic variability during the life cycle of T. crassus. See text for details.



Current environment

Past environment: previous stage

Past environment: prior definitive host

Genetic variability

effects of current environment on egg and coracidium are assumed to make a relatively small contribution since the main effect would probably arise from temperature fluctuations, and coracidia within a given area of lake or stream would probably experience similar major patterns of temperature change. However, a greater effect of current environment is shown for parasitic stages, where the environment is another animal with individual variability in physiological conditions which in turn affect its parasites (Fig. 33). It is assumed that accumulated environmental effects at one stage add to variability at subsequent stages, at least for characteristics such as growth which are expressed throughout the life cycle. The effects of the definitive host individual from which a lineage originated are given separate status based on the results of previous chapters. This source of variability is shown as relatively important for eggs and coracidia until the point of copepod infection. Experiments were not continued past the copepod host in my study but it is assumed that the effect of previous definitive host declines in later life cycle stages (Fig. 33).

The phenotypic variability that natural selection acts on results from genetic and current and past environmental effects that change in relative contribution throughout the life cycle. Understanding the nature of these changes might clarify the selection pressures that produced a high degree of life cycle synchronization in T. crassus. However, the complexity of parasite life cycles and inability to maintain most species in the laboratory will make determination of the genetic basis of their life-history characteristics difficult.

SITE SPECIFICITY OF THE ADULT

Some degree of site specificity within or on a host is virtually a universal attribute of parasitic organisms (Crompton 1973; references in chapter 2 and citations therein) and is usually attributed to resource requirements of the parasite or benefits resulting from intraspecific contact, such as reproduction.

The adaptive value of site selection by T. crassus is not clear. Attachment sites were usually clumped and this clumping induced a pathological response in the host that seemed capable of ultimately dislodging the parasite. However a large portion of the host's intestine was suitable for attachment and the pathological response could be reduced by greater dispersal of attachment sites within the intestine. There must be some substantial advantage to this behavior to counteract the seemingly considerable disadvantages.

(1) Rohde (1979) considers that site specificity promotes intraspecific contact for reproduction. However, T. crassus is a long worm and strobilae of different worms can easily come into contact even when attachment sites differ.

(2) It is possible that since T. crassus has a large scolex and hooks, damage to the host's intestine is unavoidable, and there may have been selective pressure for aggregation of attachment sites so that only a small area of the host's intestine is heavily damaged as opposed to more extensive, low-level pathology. New generations of recruits would encounter intestines with large areas of normal surface in which to establish, and the heavily-damaged areas vacated by the

previous generation could heal.

(3) It is interesting to speculate that this behavior is somehow involved in the synchronization of the reproductive behavior of T. crassus. The lesion caused by the parasite is well-vascularized so fluid leakage into the lumen is possible (chapter 1). If the cue initiating parasite maturation (which as yet is unknown) is a component of the blood or tissue fluids of the host, T. crassus would be exposed to it at high concentrations.

This study found that a small amount of variability in many characteristics of the adult worm was related to attachment site, but since natural infections were evaluated these differences could be attributable to age differences among worms. There was no conclusive evidence that particular attachment sites had a direct effect on fitness of T. crassus.

CONCLUDING REMARKS

Variability is a fundamental concept in biology since it allows populations to adapt to changing environmental conditions and to track shifting resource bases. Unfortunately, for most species of parasites we know more about variability in taxonomic characters than in life-history characteristics that affect their transmission. Furthermore, the resource base has been examined for relatively few species. My study evaluated the resource base of T. crassus in the definitive and first intermediate hosts, and used that as a basis for interpreting

the nature and extent of variability in life-history characteristics of this species. Some of the discussion in this thesis on the relationship between host and parasite ecology, parasite variability, and parasite transmission, was speculative since major gaps in our basic knowledge about parasites and host-parasite relationships emerged.

The use of natural infections as a source for T. crassus introduced a number of variables that could not be entirely accounted for such as age or past history of the individuals recovered from pike. This is an inherent problem affecting studies on naturally-acquired parasite infections but there were benefits. Natural infections allowed the examination of a diverse array of helminth communities that could not be generated in the laboratory. Furthermore, many objections that can be raised concerning the use of natural infections to assess parasite life-history characteristics were overcome by the highly-synchronized life cycle of T. crassus. Lifetime parasite fecundity could be measured. Variability observed in characteristics of adults or offspring could not be dismissed as an artifact of examining some individuals that would have matured at a later date under natural conditions, since T. crassus infrapopulations were examined in their terminal phase of existence.

Our understanding of the life cycle of T. crassus cannot be considered complete. In particular, many details of predator-prey interactions remain largely undocumented, although much of the discussion in this thesis, and the predator prey model I developed to explain maturation of T. crassus, suggested that these events are

important in transmission. More importantly, I believe that this study has made a significant contribution to our understanding of transmission of parasites with complex life cycles by evaluating the extent and sources of individual variability in parasite life-history characteristics and exploring the relationship of a parasite with several of the abiotic and biotic environments it encounters during the life cycle.

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Appendix I

Mean abundances, standard deviations, and ranges of abundances for helminths in 17 collections of northern pike in Manitoba. Abundance was the number of individuals per host, including uninfected hosts.

Appendix IA. Mean abundance, standard deviation, and range of abundance for cestodes in 17 collections of northern pike in Manitoba.

Code[a]	No. pike	T. crassus	T. nodulosus	P. pinguis
HJn81	8	-	14.4 ± 8.31 [2-26]	507 ± 154 [268-708]
HJ181	22	-	16.3 ± 25.8 [0-92]	65.0 ± 73.2 [5-198]
QJn81	11	0.455 ± 1.04[b] [0-3]	2.00 ± 3.00 [0-10]	96.7 ± 53.9 [15-220]
FAp81	19	8.00 ± 7.34 [0-26]	0.842 ± 1.80 [0-7]	187 ± 169 [1-621]
FAp82	8	16.5 ± 22.5 [0-67]	0.375 ± 0.744 [0-2]	192 ± 150 [16-395]
FAp83	20	9.70 ± 16.6 [0-72]	4.40 ± 11.1 [0-50]	124 ± 130 [2-435]
FAp84	14	11.3 ± 12.9 [0-40]	1.21 ± 2.29 [0-7]	111 ± 132 [7-442]
LJn81	13	65.8 ± 45.1 [10-157]	2.54 ± 4.33 [0-12]	210 ± 198 [16-587]
LJ181	11	19.5 ± 24.6 [0-82]	2.18 ± 3.28 [0-9]	21.7 ± 14.0 [8-52]
LAu81	5	24.4 ± 20.4 [4-48]	5.60 ± 7.73 [0-19]	54.8 ± 45.0 [4-109]
CJn81	6	47.0 ± 30.1 [10-101]	9.83 ± 23.1 [0-57]	46.8 ± 82.0 [3-213]
CJ181	58	102 ± 56.3 [20-268]	1.81 ± 6.86 [0-42]	53.8 ± 106 [1-759]
CAu81	20	91.4 ± 27.4 [31-139]	0.650 ± 1.09 [0-4]	31.6 ± 62.7 [1-280]

cont'd

Mean abundance, standard deviation, and range of abundance for cestodes in 17 collections of northern pike in Manitoba, continued.

Code[a]	No. pike	T. crassus	T. nodulosus	P. pinguis
CMr82	12	77.9 ± 30.8 [32-144]	0.500 ± 0.798 [0-2]	119 ± 207 [8-685]
CJn82	25	46.2 ± 32.1 [0-116]	2.08 ± 5.08 [0-19]	65.3 ± 89.6 [1-390]
CJl82	39	68.7 ± 48.1 [0-192]	0.436 ± 1.05 [0-4]	58.1 ± 89.0 [0-541]
CAu82	47	70.0 ± 50.2 [13-248]	1.72 ± 8.31 [0-57]	41.8 ± 69.3 [0-371]

[a] Collection site (H, Heming L.; Q, Quigly L.; F, Falcon L.; C, Channel site on Southern Indian L.; L, Long Bay site on SIL); Month; Year.

[b] $\bar{X}$ ± SD [minimum-maximum]

Appendix IB. Mean abundance, standard deviation, and range of abundance for nematodes in 17 collections of northern pike in Manitoba.

Code[a]	No. pike	*R. acus*	Code[a]	No. pike	*R. acus*
HJn81	8	61.5 ± 62.8[b] [5-198]	CJn81	6	16.8 ± 6.62 [10-27]
HJ181	22	2.68 ± 3.47 [0-15]	CJ181	58	34.1 ± 24.5 [0-103]
QJn81	11	23.7 ± 20.9 [3-77]	CAu81	20	28.8 ± 22.3 [5-91]
FAp81	19	19.1 ± 26.2 [0-79]	CMr82	12	32.8 ± 19.3 [7-70]
FAp82	8	12.9 ± 15.9 [0-47]	CJn82	25	17.8 ± 15.5 [0-55]
FAp83	20	14.7 ± 25.3 [0-108]	CJ182	39	15.2 ± 17.7 [0-100]
FAp84	14	10.9 ± 13.9 [0-48]	CAu82	47	22.5 ± 20.2 [0-90]
LJn81	13	65.8 ± 45.1 [2-80]			
LJ181	11	3.54 ± 4.37 [0-13]			
LAu81	5	7.00 ± 7.58 [1-20]			

[a] See Appendix IA for definitions of codes.

[b] $\bar{X}$ ± SD [minimum-maximum]

Appendix IC. Mean abundance, standard deviation, and range of abundance for acanthocephalans in 17 collections of northern pike in Manitoba.

Code[a]	No. pike	*E. leidyi*	*E. salmonis*	*N. tenellus*	*L. thecatus*
HJn81	8	-	-	-	-
HJ181	22	-	-	-	-
QJn81	11	-	-	-	-
FAp81	19	-	-	1.58 ± 3.53 [0-13]	0.90 ± 2.02 [0-6]
FAp82	8	-	-	0.50 ± 0.76 [0-2]	11.0 ± 19.5 [0-44]
FAp83	20	-	-	0.20 ± 0.52 [0-2]	3.25 ± 9.20 [0-38]
FAp84	14	-	-	0.071 ± 0.27 [0-1]	3.93 ± 8.49 [0-30]
LJn81	13	1.69 ± 1.89 [0-6]	0.077 ± 0.28 [0-1]	-	-
LJ181	11	-	-	-	-
LAu81	5	-	-	-	-
CJn81	6	0.83 ± 2.04 [0-5]	-	-	-
CJ181	58	3.31 ± 4.82 [0-20]	0.28 ± 1.01 [0-7]	-	-
CAu81	20	0.35 ± 0.81 [0-3]	0.30 ± 0.66 [0-2]	-	-

cont'd

Mean abundance, standard deviation, and range of abundance for acanthocephalans in 17 collections of northern pike in Manitoba, continued.

Code[a]	No. pike	*E. leidyi*	*E. salmonis*	*N. tenellus*	*L. thecatus*
CMr82	12	1.17 ± 0.937 [0-3]	0.083 ± 0.289 [0-1]	-	-
CJn82	25	1.36 ± 3.60 [0-15]	0.040 ± 0.200 [0-1]	-	-
CJ182	39	2.26 ± 4.74 [0-26]	0.513 ± 1.68 [0-10]	-	-
CAu82	47	0.13 ± 0.397 [0-2]	0.021 ± 0.146 [0-1]	-	-

[a] See Appendix IA for definitions of codes.

[b] $\bar{X}$ ± SD [minimum-maximum]

Appendix II

Life-history characteristics of lineages of T. crassus collected in 1983-1984 and used for factor analysis in chapter 7.

Appendix IIA. Life-history characteristics of lineages of T. crassus collected in 1983.

Lineage	Host	A	B	C	D	E	F	G	H	I	J
8	504	41	880	0.94	2.20	0.17	0.85	6.4	0.93	0.57	0.68
9	504	60	2020	0.80	2.24	0.43	0.88	6.8	0.86	0.90	0.65
10	504	61	1780	0.80	2.42	0.21	0.86	6.0	0.76	1.01	0.80
11	504	37	810	0.96	2.25	0.23	0.79	5.0	0.90	1.37	0.79
12	506	56	4400	0.50	2.38	0.28	0.65	5.9	0.93	1.07	0.43
14	507	50	656	0.93	2.34	0.18	0.57	6.0	0.87	0.69	0.26
15	507	86	312	0.95	2.27	0.10	0.36	3.8	0.93	0.99	0.00
17	507	82	1940	0.94	2.43	0.24	0.56	7.9	0.97	0.90	0.26
18	507	54	1260	0.88	2.39	0.18	0.54	3.8	0.90	0.83	0.47
41	517	76	408	0.40	2.43	0.25	1.00	4.4	0.86	0.76	0.37
52	520	17	920	0.85	2.09	0.15	0.93	6.3	0.90	0.84	0.45
54	520	67	1056	0.98	2.02	0.21	0.69	4.7	0.87	1.11	0.60
57	520	64	704	0.93	2.20	0.23	0.65	7.8	0.86	1.16	0.42
58	520	33	820	1.00	2.28	0.18	0.55	3.7	0.93	0.57	0.29
62	520	54	382	0.98	2.01	0.27	0.85	5.0	0.86	0.66	0.32
63	520	62	218	0.95	2.05	0.20	0.67	2.0	0.97	0.81	0.26
66	521	41	656	0.98	2.45	0.15	0.21	6.9	0.87	0.79	0.41
118	527	31	260	0.84	2.33	0.13	0.25	1.2	0.97	0.25	0.00

Note: A, adult mass, mg; B, fecundity, eggs x 10^{-3}; C, proportion of eggs hatching; D, median egg hatch time, ln(days); E, variance of egg hatch time; F, proportion of copepods infected; G, intensity in copepods; H, proportion of copepods surviving 10 days; I, adjusted procercoid volume, nl; J, proportion of procercoids differentiated.

Appendix IIB. Life-history characteristics of lineages of *T. crassus* collected in 1984.

Lineage	Host	A	B	C	D	E	F	G	H	I	J
3	530	56	910	0.96	1.98	0.24	0.88	6.6	0.83	0.37	0.32
6	530	48	790	0.90	1.95	0.18	0.91	5.9	0.84	0.36	0.30
7	530	36	420	0.93	2.13	0.14	0.93	4.6	0.95	0.21	0.25
25	531	63	1100	0.90	2.14	0.19	0.73	9.0	0.68	0.46	0.21
26	531	102	1400	0.96	2.42	0.23	0.89	6.5	0.78	0.74	0.27
27	531	30	130	0.95	2.48	0.10	1.00	5.5	0.90	0.26	0.58
29	531	68	1540	0.96	2.28	0.24	0.82	8.2	0.95	0.33	0.12
30	531	23	286	0.98	2.49	0.19	0.90	6.5	0.76	0.36	0.21
32	531	84	1780	0.98	2.35	0.18	0.93	7.1	0.81	0.40	0.16
33	531	41	730	0.96	2.39	0.21	0.88	8.2	0.80	0.32	0.27
35	531	40	214	0.99	2.42	0.16	0.92	7.8	0.70	0.23	0.18
37	532	124	1680	0.94	2.14	0.15	0.91	6.0	0.93	0.36	0.27
51	535	69	375	0.95	2.01	0.05	1.00	7.2	0.85	0.40	0.41
55	535	76	256	0.99	2.07	0.10	1.00	4.8	0.75	0.20	0.12
60	537	113	2840	0.96	1.86	0.17	0.85	7.8	0.80	0.48	0.18
61	537	97	2840	0.94	1.94	0.23	0.83	6.8	0.80	0.32	0.15
62	537	50	360	0.96	2.01	0.09	0.92	7.9	0.90	0.36	0.28
63	541	46	690	1.00	2.11	0.13	1.00	10.6	0.75	0.51	0.47
64	541	48	605	0.97	2.08	0.14	1.00	7.9	0.63	0.18	0.14
69	541	23	144	0.99	2.14	0.10	1.00	6.0	0.80	0.51	0.46

Note: A, adult mass, mg; B, fecundity, eggs x 10^{-3}; C, proportion of eggs hatching; D, median egg hatch time, ln(days); E, variance of egg hatch time; F, proportion of copepods infected; G, intensity in copepods; H, proportion of copepods surviving 10 days; I, adjusted procercoid volume, nl; J, proportion of procercoids differentiated.