THE BIOLOGY OF *Cystidicola farionis* FISCHER, 1798 (NEMATODA: CYSTIDICOLIDAE) IN SALMONID FISHES

BY

ALAN J. DEXTRASE

A THESIS

PRESENTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF BIOLOGY

LAKEHEAD UNIVERSITY

THUNDER BAY, ONTARIO

JANUARY 1987

ISBN 0-315-39583-4

Abstract

Morphological studies, experimental cross-infections and host specificity data suggest that the species of swimbladder nematode which matures in *Coregonus clupeaformis*, from inland lakes is not distinct from *Cystidicola farionis* which matures in *Coregonus artedii* and other salmonids from Lakes Superior and Nipigon. Adult worms in *Coregonus clupeaformis* are morphologically indistinguishable from those in other hosts. However, eggs of worms in *Coregonus clupeaformis* have predominantly lateral filaments, while those in other hosts have predominantly polar filaments. Larvae which accumulate but do not mature in the swimbladder of *Coregonus clupeaformis* from Lake Nipigon, grew and developed toward maturity in hatchery-reared *Coregonus clupeaformis* of Lake Simcoe origin. One, mature, male worm was found 77 days after experimental infection. Only female worms containing unshelled eggs were found after 120 days. This suggests that the development of *C. farionis* may depend on host strain. Uncertainty remains as to whether variations in egg morphology are host-induced or due to genetic differences of the worms involved.

Filaments on the eggs of *C. farionis* readily adhere to the setose areas on the appendages of amphipod intermediate hosts. Presumably, this increases the probability of the intermediate host ingesting eggs. Eggs stored in water or saline for periods of two weeks or more do not hatch in the gut of amphipods. Only eggs recently released from female worms hatch and develop.

Mature swimbladder nematodes were found in *Coregonus clupeaformis* from all of seven inland lakes examined in northwestern Ontario. *Coregonus artedii* was present in three of these lakes but was not infected, perhaps due to the lack of amphipod intermediate hosts in its diet. *Cystidicola farionis* matures in *Salvelinus*

namaycush from Pettit Lake, Ontario and has eggs resembling those from worms in *Coregonus clupeaformis* from the same lake. *Prosopium coulteri* from Lake Superior are infected with *C. farionis* (new host record) but are not considered to be a suitable host for the worm as no mature worms were found. Infections in *Coregonus clupeaformis* from Pettit Lake and in *Salmo gairdneri* from Lake Superior exhibited similar seasonal periodicity with largest worm numbers occurring in the spring.

The cephalic structures of *C. farionis*, and its close relative, *C. stigmatura* were studied using a scanning electron microscope and are compared with those of other cystidicolids.

Acknowledgements

I would like to express my sincere gratitude to Dr. Murray W. Lankester for his support and assistance with all aspects of my thesis research. His advice and patience is greatly appreciated. I am also grateful to Dr. Lankester for financial support from his NSERC operating grant.

I wish to thank Drs. R. Freitag and W. T. Momot for their advice and editorial assistance. I am grateful to Drs. R. Freitag, W. T. Momot and J. P. Ryder for allowing me to borrow equipment. The provision of aquatic laboratory facilities by Dr. W. T. Momot is greatly appreciated.

I wish to thank Al MacKenzie for his guidance on the scanning electron microscope and his help with the photography. Several people provided valuable technical assistance in the laboratory including K. Armstrong, H. Ball, M. Fruetel, G. Morgan and P. L. Wong. The advice on statistical analysis from L. Hauta is greatly appreciated.

Much of my field work would not have been possible without the support of several people from the Ontario Ministry of Natural Resources. I thank Messrs. P. Ryan, J. George, W. MacCallum, K. Cullis, R. Borecki and their employees for providing invaluable assistance. I am grateful to J. Tost for ageing lake whitefish.

Numerous people were helpful in obtaining swimbladders and nematodes during this study. I would like to thank Dr. G. Black, Dr. S. Boutin and G. Persson for collecting swimbladder nematodes from northern and western Canada. Dr. J. C. Holmes kindly provided nematodes from Cold Lake, Alta. I am grateful to Dr. E. J. Crossman and C. W. Nelson for loaning me museum specimens of pygmy whitefish from Lake Superior. Mr. R. Lindeman's knowledge concerning amphipods in the area

was invaluable and is gratefully acknowledged. I appreciate the cooperation and interest of many anglers who fished the streams along the North Shore; J. George, G. Johnson, N. Sakamoto and Dr. G. Ozburn were especially helpful.

Finally, I would like to say thanks to many friends whose encouragement has been very helpful. Special thanks are due to Helen Ball for her love and support. Thank you for putting up with me. The constant support and encouragement from my parents is greatly appreciated.

This thesis is dedicated to the memory of my father who sparked my interests in biology when I was very young.

Table of Contents

List of Figures

List of Tables

Introduction

Nematodes of the genus *Cystidicola* Fischer, 1798 (Habronematoidea: Cystidicolidae) are parasitic in the swimbladder of salmonid fishes. Two valid species are presently recognized (Black 1983a). *Cystidicola stigmatura* (Leidy, 1886) occurs in charr (*Salvelinus* spp.) from North America and utilizes the oppossum shrimp (*Mysis relicta* Löven) as an intermediate host (Smith and Lankester 1979). *Cystidicola farionis* Fischer, 1798, occurs in a variety of salmonids from Eurasia and North America, and uses amphipods as an intermediate host (Smith and Lankester 1979). The two species are most easily distinguished by the morphology of their eggs. The eggs of *C. farionis* are filamented while those of *C. stigmatura* have lateral lobes.

Reports on the occurrence of *C. farionis* in various hosts are numerous (see Margolis and Arthur 1979), but its biology has only recently received attention (Smith and Lankester 1979; Black and Lankester 1980; Lankester and Smith 1980). Development of *C. farionis* is retarded in some hosts such that worms rarely reach maturity. Lake whitefish (*Coregonus clupeaformis* (Mitchill)) from Lake Nipigon are often infected with large numbers of *C. farionis* yet no mature worms have been found (Lankester and Smith 1980). Immature worms are also common in lake whitefish from Lake Superior although a small proportion are mature in some individuals. Lake herring (*Coregonus artedii* (LeSueur)) and other salmonids from both of these lakes harbour large numbers of mature *C. farionis*. Conversely, lake whitefish from Lake Huron and several inland lakes are infected with mature worms (Lankester and Smith 1980), while lake herring in the same waters are not infected (Watson and Dick 1979) or have only immature worms (Leong and Holmes 1981).

Worms which mature in lake whitefish have eggs with mainly lateral filaments while eggs of worms which mature in other salmonids have predominantly polar filaments (Lankester and Smith 1980). The apparent differences in egg morphology and varying host specificity led Lankester and Smith (1980) to speculate that the worm which matures in the swimbladder of lake whitefish may represent a new species of *Cystidicola*. In the present study, the morphology of adult worms and eggs, host specificity data and experimental cross-infections were used as a test of this hypothesis.

Methods and materials

The swimbladders of fishes examined for *Cystidicola farionis* were collected between spring 1983 and winter 1985. Rainbow trout (*Salmo gairdneri* Richardson), coho salmon (*Oncorhynchus kisutch* (Walbaum)), and rainbow smelt (*Osmerus mordax* (Mitchill)) were taken by anglers in rivers along the north shore of Lake Superior, east from Thunder Bay to Terrace Bay, Ont. Rainbow trout, lake trout (*Salvelinus namaycush* (Walbaum)), chinook salmon (*Oncorhynchus tshawytscha* (Walbaum)), lake whitefish, lake herring, and round whitefish (*Prosopium cylindraceum* (Pallas)) were taken by anglers and commercial fishermen from Thunder Bay and Black Bay of Lake Superior. Pink salmon (*Oncorhynchus gorbuscha* (Walbaum)) were taken in a seine net in Blind Creek, Thunder Bay (48°29'N,89°04'W). Pygmy whitefish (*P. coulteri* (Eigenmann and Eigenmann)), collected in the summer of 1955 from Keweenaw Bay of Lake Superior and preserved in 70 % ethanol, were borrowed from the University of Michigan Museum of Zoology, Ann Arbour, Michigan (UMMZ 167956). Other pygmy whitefish collected from Keweenaw Bay in 1955 and from Quebec Harbour of Lake Superior in 1963 were borrowed from the Royal Ontario Museum, Toronto, Ontario (ROM 16844 and ROM 22497, respectively). Coho salmon were taken by anglers from Lake Ontario near Port Credit, Ontario. Rainbow trout, coho salmon and chinook salmon were taken by commercial fishermen in the North Channel of Lake Huron. Lake whitefish and lake herring were taken by commercial fishermen and in gill nets set by the author with the Ontario Ministry of Natural Resources (OMNR) from Lake Nipigon. Samples from several smaller, inland lakes in northwestern Ontario included lake whitefish and lake herring from Lac Des Mille Lacs (48°52'N,92°28'W), Greenwater Lake (48°34'N,90°26'W), and Pettit Lake

(48°57'N,92°16'W) and lake whitefish from Sandford Lake (49°08'N,91°41'W), Little Dog Lake (48°39'N,89°30'W), Mount Lake (49°02'N,92°10'W), and Wakitchen Lake (49°00'N,92°13'W). Swimbladder nematodes were examined from lake whitefish of Cold Lake, Alta. (54°33'N,110°05'W) and Slave River, NWT (61°18'N,113°40'W). One arctic charr (*Salvelinus alpinus* (L.)) was examined from Willow Lake, NWT (68°20'N,107°45'W). In addition, swimbladder nematodes were examined from lake trout of Pettit Lake, Mount Lake, Icarus Lake, Ont. (48°13'N,90°34'W), Simmons Lake, BC (59°11'N,129°46'W) and Aishihik Lake, YT (61°25'N,137°15'W). Scientific and common names of fishes follow those given by Bailey et. al. (1980).

Fish were identified and fork length, weight and sex were recorded. Swimbladders were removed and dissected fresh in 0.7% physiological saline solution (7 g of NaCl in 1000 ml of distilled water). For museum specimens, existing incisions in fish were extended and the swimbladders removed. Specimens were returned to their respective collections. Swimbladders were examined using a dissecting microscope at 6 to 25 X. Worms were fixed in hot glycerin-alcohol (10% glycerin in 70% alcohol) and cleared in glycerin. Worms from swimbladders that had been previously fixed were placed in glycerin-alcohol at room temperature and cleared in glycerin. Female worms with shelled eggs in the uterus and male worms with sperm in the *vas deferens* were considered mature; all others were considered immature. Sex of third-stage larvae was determined by the appearence of the gonad; the male gonad is convoluted and extends anteriorly to the middle of the body while the female gonad is straight and extends into the anterior half of the body (Black and Lankester 1980). All drawings and measurements were made with the aid of a *camera lucida.* Common parasitological terms are used as defined by Margolis et al. (1982).

Otoliths and pelvic fin rays were used to age lake whitefish from Pettit Lake.

Stomachs from these fish were preserved in 5 percent formalin and later examined to determine if there were seasonal differences in feeding habits which might explain seasonal parasite differences. Stomachs were also examined from three lake herring and two lake trout from Pettit Lake.

Experimental cross-infections with *C. farionis* were attempted between lake whitefish and rainbow trout. Worms from Pettit Lake lake whitefish were raised in amphipods and given to hatchery-reared rainbow trout and lake whitefish. Worms from Lake Superior rainbow trout were raised in amphipods and given to lake whitefish and rainbow trout. Fish were obtained from hatcheries and fed a commercially prepared, pelletized, fish food. They were held in 350 l polyethelene tanks with a constant flow (2 l/min) of dechlorinated water (8-21°C).

Amphipods used in experiments were identified using Bousfield (1958). Laboratory raised *Hyalella azteca* (Saussure) and *Gammarus fasciatus* Say collected from various localities in and around the city of Thunder Bay, Ont., were used in experimental work. Amphipods were held in 60 l glass aquaria or 60 l styrofoam coolers containing aerated dechlorinated water. Natural substrate was placed in the tanks along with glass wool, natural vegetation and decaying wood (Smith 1978). The water in the tanks was replenished weekly with either distilled water or dechlorinated water. Amphipods were fed a diet of Tetra Min staple fish food flakes supplemented with dried, deciduous leaf litter (Smith 1978).

Eggs were removed from live female *C. farionis* by three different methods. Mature female worms were placed in a settling flask containing distilled water for 24 hours. Eggs released via the vulva of females were stored in distilled water, dechlorinated water or saline at 6°C for up to 4 months. A second technique involved dissecting eggs from the distal one third of the uterus in saline. Eggs collected in this manner were fed to amphipods within 48 hours of their removal

from female worms. Eggs were more efficiently recovered from females when large batches of worms were chopped in a blender in saline.

Amphipods were exposed to eggs which had been recently removed from live worms and to eggs which had been stored in various media at 6°C for different time periods. Amphipods were deprived of food for 24 hours pre-exposure. *Hyalella azteca* were exposed for 2 to 18 hours to uncounted numbers of eggs in 8 ml concave infection dishes containing water from their holding tanks. A small amount of finely ground Tetra Min staple fish food was added to each dish to stimulate feeding and to act as an indicator of amphipod feeding activity. The larger *G. fasciatus* were similarly exposed in 350 ml finger bowls. Amphipods were dissected using a stereomicroscope at various intervals after exposure to eggs. The lumen of the intestine was examined for ingested eggs and hatched first-stage larvae as well as Tetra Min flakes. The haemocoel and remaining tissues were examined for the presence of *C. farionis* larvae.

Amphipods, exposed 5 to 8 weeks earlier to *C. farionis* eggs, were fed to lake whitefish and rainbow trout in order to establish experimental infections. Fish were isolated within their holding tanks to ensure that each individual ingested equal numbers of amphipods. A plexiglass chamber (30 cm x 30 cm x 65 cm), open at top and bottom, was placed over an individual fish and amphipods were introduced from the top. Each fish was removed from the chamber 30 minutes after it had eaten the last amphipod. Fish were sacrificed by cervical dislocation at various intervals after exposure. Swimbladders were removed and examined for *C. farionis*.

Rainbow trout and lake whitefish were also infected by feeding them a section of an earthworm, *Lumbricus terrestris*, into which third-stage larvae of *C. farionis* had been injected. Larvae were obtained from the swimbladders of Lake Nipigon lake whitefish in March, 1985. Earthworms were cut into 2 cm sections and one

end was tied off tight with thread. Batches of 100 larvae were drawn into a 3 cc syringe through a 16 gauge needle in approximately 0.1 cc of saline. The untied end of the earthworm section was placed over the needle of the syringe. This end was tied off with thread around the needle and held taut while the contents of the syringe were injected into the earthworm. The section of earthworm was removed from the needle and the thread was tied off. Each fish was given one section of earthworm containing 100 larvae in the plexiglass chamber. Some fish would not feed within the chamber. In this case the earthworm was introduced into the main tank and the fish consuming it was identified and isolated for 30 minutes in the chamber.

The potential of infected rainbow smelt to transmit *C. farionis* to large piscivorous salmonids was investigated. Smelt were captured in McVicar Creek (48°26'N,89°13'W), Thunder Bay, Ont. between April 24 and May 2, 1984. Twenty were frozen and later examined to determine numbers of potentially infective, third-stage, *C. farionis* larvae present. The stage of larval development was determined by examining *en face* preparations (Anderson 1958), by the degree of gonad development and by the presence or absence of a cuticular projection at the base of the tail (Black and Lankester 1980). An additional 20 smelt were held live in a 60 l styrofoam cooler containing aerated, dechlorinated water. One to three live smelt were fed daily to a large, hatchery-reared rainbow trout for a period of eleven days. The rainbow trout was examined for swimbladder nematodes 148 days after it had eaten the last smelt.

For scanning electron microscopy (SEM), live worms were fixed overnight in buffered glutaraldehyde (10 ml of 25% glutaraldehyde in 95 ml of phosphate buffer=7 parts 7.1 g of Na_2HPO_4 in 500 ml distilled water: 3 parts 7.8 g of NaH_2PO_4 in 500 ml distilled water). Worms were rinsed in phosphate buffer and post-fixed overnight

in 4% osmium tetroxide (500 mg of OsO_4 in 50 ml of phosphate buffer). Post-fixed worms were rinsed with distilled water and dehydrated in a graded acetone series (50%, 70%, 90%, 95%, 100%, 100% acetone in distilled water). Dehydrated worms were critical point dried with CO_2 in a Sorval critical point drying apparatus. Dried specimens were mounted on aluminum tent stubs and coated with gold in a Fullam EMS-76M sputter coater. Worms were viewed at 15kV with a Cambridge S600 SEM. Amphipods were prepared in the same manner.

Variability in the position and number of filaments on eggs of *C. farionis* in different host species was investigated using the SEM. Ten mature female worms from each host species were frozen in saline. Worms were later thawed and mature eggs dissected from the vagina and distal 1 mm of each uterine branch. Eggs from the ten female worms were mixed and pipetted onto a Nuclepore membrane filter (pore diameter 0.2 m) in a Millipore vacuum filtering apparatus. Eggs were rinsed in distilled water, fixed in 5% AFA (90 ml of 70% alcohol, 5 ml of glacial acetic acid, 5 ml of 100% formalin) for three hours and rinsed again in the filtering apparatus. Filter papers with attached eggs were placed into a petri dish of distilled water and cut to stub size with a cork borer (diameter 14 mm). After dehydration in a graded acetone series (30%, 50%, 70%, 90%, 100%, 100%), eggs were critical point dried, mounted on stubs and coated with gold. Coated specimens were viewed at 7.5 kV. Thirty eggs were randomly chosen from each sample. Individual eggs were photographed at 500 X and 1000 X. Counts of lateral and polar filaments were made from 35 mm photomicrographs.

The total number of eggs in each of five *C. farionis* from Lake Superior rainbow trout and five *C. stigmatura* from Icarus Lake lake trout was estimated. Eggs in two measured body sections cut from fixed, female *C. farionis* were counted and an estimate of total numbers made by multiplying the mean number of eggs by

the length of the body containing uterus. This method was used since it is extremely difficult to remove the uterus intact from *C. farionis*. The entire reproductive tract was dissected out of fixed, female *C. stigmatura* and eggs were counted in six measured sections.

All statistical tests follow Steele and Torrie (1980). Analysis of variance and covariate analysis were used to examine the effects of fish sex, age, and length, and season of capture on the intensity of *C. farionis* infections and the proportion of worms reaching sexual maturity. Analysis of variance was used to examine the effect of host species on the distribution and lengths of filaments on nematode eggs. Chi-square and Scheffe's range tests were computed when applicable. The minimum level for statistical significance was $P<0.05$ for all tests. Mean values given in the text are followed by standard error.

Results

Examination of naturally infected fish

Inland lakes

Cystidicola farionis was found in lake whitefish from all seven inland lakes sampled (Table 1). The prevalence of infection ranged from a high of 100% in Little Dog Lake to 37% in Lac Des Mille Lacs. The number of worms in each infected fish was usually low ($\bar{x}$=15). Considerable variation existed between lakes in the mean percentage of mature worms per infected fish (Table 1). Length and sex of fish had no effect on intensity of infection or the mean percentage of mature worms in lake whitefish collected from Greenwater (intensity $F_{2,84}$=0.49, maturity $F_{2,84}$=1.01), Mount (intensity $F_{2,27}$=0.32, maturity $F_{2,27}$=2.12) and Wakitchen (intensity $F_{2,26}$=0.70, maturity $F_{2,26}$=0.39) lakes. The effect of season could not be tested as samples were collected at one time only from each of these lakes.

Lake herring were examined from three lakes from which infected lake whitefish were obtained. None contained swimbladder nematodes (Greenwater Lake, n=26, fork length 11-32 cm, examined summer 1983; Lac Des Mille Lacs n=15, fork length unknown, summer 1984; Pettit Lake, n=4, fork length 11-13 cm, fall 1984). No amphipods were present in three lake herring stomachs examined from Pettit Lake.

Sufficient infected lake whitefish were available from Pettit Lake to test the possible effect of season of capture on prevalence, intensity and the proportion of worms that had reached sexual maturity (Table 2). Fish collected in the fall (Nov. 1, 1983 and Oct. 28-30, 1984) were gill-netted on spawning shoals in 1 to 3 m

Table 1. *Cystidicola farionis* in lake whitefish (*Coregonus clupeaformis*) from inland lakes in northwestern Ontario, 1983-1985.

Lake	Season sampled*	n	Length of fish	Prevalence	Mean intensity	Mean % mature worms per infected fish
Greenwater Lake	Su83	87	38.3±0.66[+] (17.5-53.9)	92	23±2.0[++]	17
Lac Des Mille Lacs	Su83,Su84	154	43.0±0.84 (35.3-52.0)	37	2±1.2	85
Little Dog Lake	Su85	2	45.1,47.1	100	2,3	50
Mount Lake	F83	30	40.3±0.49 (36.3-46.3)	63	11±2.5	35
Pettit Lake	F83,S84,F84	133	38.4±0.48 (17.3-47.7)	69	19±2.1	39
Sandford Lake	F83	7	?	?	?	100
Wakitchen Lake	F83	29	40.5±0.51 (36.1-50.0)	62	7±3.0	21

*S,April-May; Su,June-August; F,September November, followed by year.

[+]Mean fork length (cm) ± SE, subtended by range in parentheses.

[++]Mean ± SE.

Table 2. *Cystidicola farionis* in lake whitefish (*Coregonus clupeaformis*) from Pettit Lake, Ontario in three different seasons.

Age of fish (years)	*n*	Length of fish	Season sampled											
			Fall 1983				Spring 1984				Fall 1984			
			n	Prevalence	Intensity	% Mature*	*n*	Prevalence	Intensity	% Mature	*n*	Prevalence	Intensity	% Mature
1 to 5	5	19.0±2.2** (17.3-22.6)	0	–	–	–	1	0	–	–	4	0	–	–
6 to 10	40	35.6±0.8 (25.1-44.7)	14	64	9±2.7+	33	13	100	22±5.7	49	13	23		0
11 to 15	55	39.9±0.4 (33.6-47.3)	19	79	19±6.8	9	31	97	29±3.9	54	5	20		80
16 to 20	12	43.6±0.7 (39.6-47.7)	3	67	28	0	9	100	24±4.6	71	0			
> 20	3	45.4±0.8 (44.1-46.9)	0				3	67	18	58	0			
All fish++	133	38.4±0.5 (17.3-47.7)	38	74	16±4.1	16	57	95	25±2.5	57	38	26	3±1.1	8

*Mean percent mature worms per infected fish.

**Mean fork length (cm) ± SE, subtended by range in parentheses.

+Mean intensity ± SE.

++Includes 2 and 16 fish caught in fall 1983 and fall 1984 respectively, which were not aged.

of water by a commercial fisherman. Fish caught in the spring (May 18-21, 1984) were taken in trap nets in 3 m of water and in gill nets set at depths around 10 m. Lake whitefish sampled were 4 to 26 years old ($\bar{x}$=11.8) and from 17 to 47 cm in fork length ($\bar{x}$=38.4 $\pm$ 0.48). Sex, age and length of lake whitefish had no significant effect on the intensity of infection or the mean percentage of mature worms per infected fish (Table 2) (intensity $F_{2,89}$=7.53; maturity $F_{2,89}$=1.98). Older fish tended to have more worms and fewer mature worms than younger fish in the fall 1983 sample, but this trend was not statistically significant. No fish younger than six years old were infected, but only five fish were examined. The prevalence of *C. farionis* and the mean percentage of mature worms per infected fish were higher in the spring than in both fall samples (prevalence X^2=2.11, d.f.=133; maturity $F_{2,89}$=21.27) (Fig. 1). The mean intensity of infection was lower in the fall of 1984 than in fall 1983, and spring 1984 ($F_{2,89}$=19.63).

There were marked differences in the feeding habits of lake whitefish collected in spring and fall of 1984 (Table 3). *Hyalella azteca*, a shallow water amphipod, was found in the stomachs of fish caught in the fall while *Pontoporeia affinis* Lindstrom, a deepwater species, was the only amphipod found in the spring sample. Pelycypods and dipterans were most common in the spring sample. Ephemeropteran nymphs, specifically those of *Hexagenia* sp., were common in fish from both seasons. No *Mysis relicta* were found in the stomach of any lake whitefish.

Four of five lake trout (fork length 42-58 cm, $\bar{x}$=51.5 $\pm$ 3.1) examined from Pettit Lake in fall of 1984 were infected with *C. farionis* (intensity 2-15, $\bar{x}$=6) of which 18 worms (75%) were mature. One lake trout (65 cm) examined in fall 1984 from adjoining Mount Lake had *C. stigmatura* (182 worms), but not *C. farionis*. There were no *M. relicta* in the stomachs of two infected lake trout examined from Pettit Lake.

Figure 1. *Cystidicola farionis* infections in lake whitefish, *Coregonus clupeaformis*, collected from Pettit Lake, Ont., in fall and spring 1983-1984.

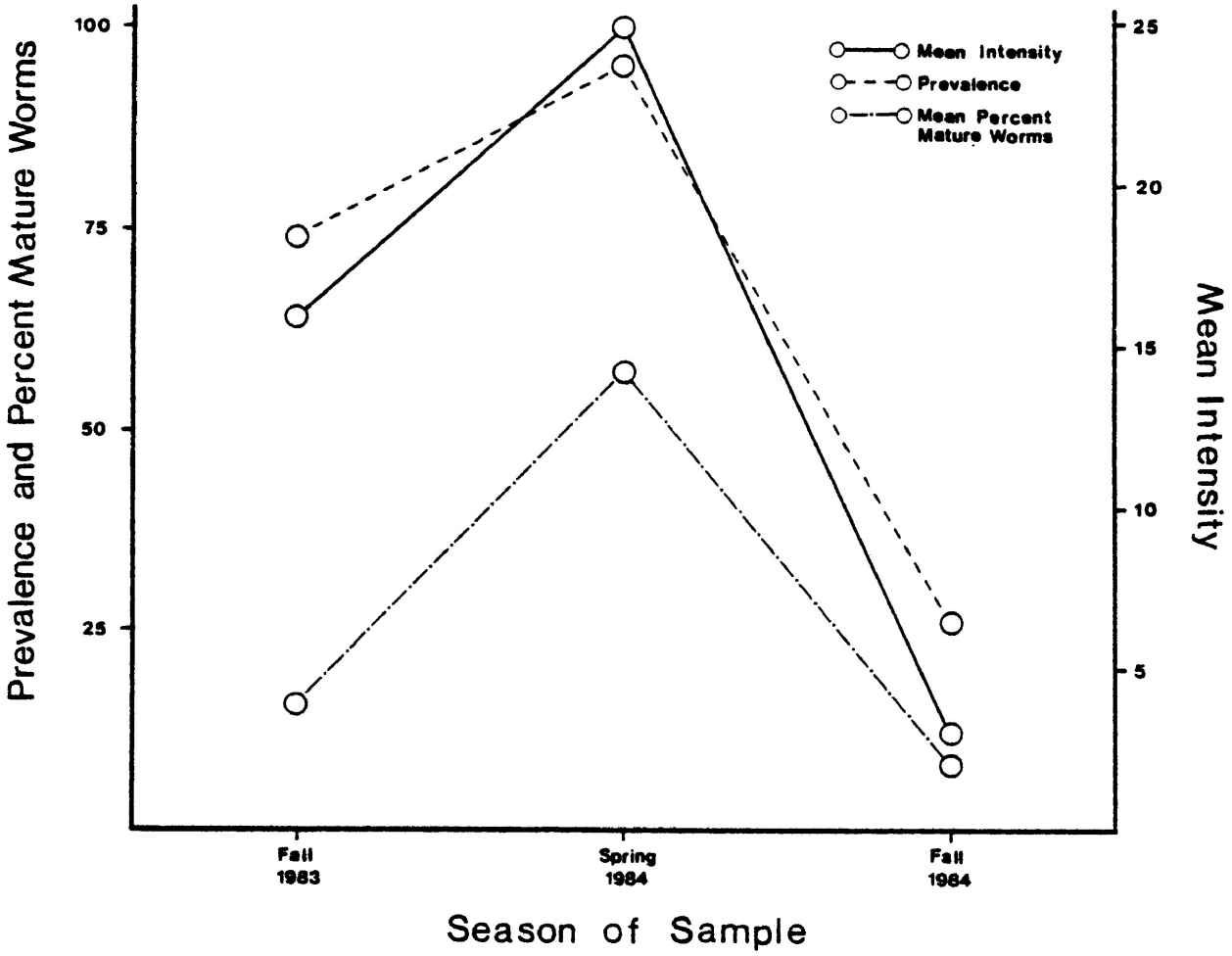

Prevalence and Percent Mature Worms
100
75
50
25
Mean Intensity
25
20
15
10
5
Mean Intensity
Prevalence
Mean Percent Mature Worms
Fall 1983
Spring 1984
Fall 1984
Season of Sample

Table 3. Stomach contents of lake whitefish (*Coregonus clupeaformis*) from Pettit Lake, Ontario, 1984.

	Season of sample	
	Spring 1984 (n=50)	Fall 1984 (n=30)
Gastropoda	22*	26
Pelycypoda	60	10
Hydracarina	16	29
Cladocera	—	16
Ostracoda	—	3
Isopoda	—	3
Amphipoda		
Hyalella azteca	—	13
Pontoporeia affinis	28	—
Ephemeroptera	76	45
Hemiptera	4	13
Megaloptera	34	3
Trichoptera	8	19
Coleoptera	6	3
Diptera (larvae)	76	29
Diptera (adults)	20	—
Hymenoptera	4	—
Fish	—	10
Empty	6	35

*Percentage occurrence.

Lakes Superior, Nipigon, Huron and Ontario

A variety of salmonids from Lakes Superior and Nipigon were infected with *C. farionis* (Table 4). Lake whitefish had only immature worms, often in large numbers. A large proportion of worms in lake herring from both lakes was mature (68% and 74% for Superior and Nipigon respectively). Twenty-three percent of worms in rainbow trout from Lake Superior were mature. Pygmy whitefish from Lake Superior had only immature worms (5-8 mm long)

Incidental examination of fish from Lake Huron revealed mature *C. farionis* in one of two rainbow trout, but one coho salmon and one chinook salmon were not infected (small sample sizes and a single sampling period limited the value of the Lake Huron data). No swimbladder nematodes were found in 10 coho salmon examined from Lake Ontario.

Sufficient infected rainbow trout were available from Lake Superior to test the possible effect of season on the prevalence and intensity of *C. farionis* infections, and the proportion of worms reaching sexual maturity. Prevalence, mean intensity and mean percentage of mature worms per infected fish were higher in spring 1983 and 1984 than in fall 1983 and 1984 (prevalence X^2=13.12, d.f.=153; intensity $F_{3,93}$=2.74; maturity $F_{2,31}$=6.83). Limited data prevented testing the effects of age, sex and length of rainbow trout on *C. farionis* infections. Fish sampled in spring 1984 were not included in the maturity sample as both immature and male worms were destroyed with female worms during egg removal in the blender.

Experimental infections

Experimental infection of amphipods

Eggs of *C. farionis* were fed to amphipods in attempts to obtain third-stage

Table 4. *Cystidicola farionis* in fishes from Lakes Superior, Nipigon, Huron and Ontario, 1983-1985.

Fish species and location	Season sampled*		Length of fish	Prevalence	Mean intensity	Mean % mature worms per infected fish
Lake Superior						
Coregonus artedii	Su83,F83	36	32.0±0.7** (22.9-39.3)	64	39±12.7+	68
Coregonus clupeaformis	Su84	7	?	43	1±0.3	0
Prosopium coulteri	Su55,Su63	35	9.9±0.3 (6.8-13.2)	20	1±0.2	0
Prosopium cylindraceum	Su83	20	33.4±0.8 (28.4-39.9)	25	7±4.4	93
Oncorhynchus gorbuscha	F83	2	?	100	54	85
Oncorhynchus kisutch	F84,F85	4		100	116±12.0	67
Oncorhynchus tshawytscha	S84,Su84	4		100	14±7.9	22
Salmo gairdneri	S83,Su83,F83,S84, Su84,F84	153	50.8±1.6 (31.0-71.0)	63	37±5.9	23++
Salvelinus namaycush	Su83	2	?	100	9	0
Osmerus mordax	S84	20	13.7±0.2	75	4±0.9	0
Lake Huron						
Oncorhynchus kisutch	F84		61.3	0		
Oncorhynchus tshawytscha	F84		49.0	0		
Salmo gairdneri	F84	2	70.3 (64.1,76.5)	50		100
Lake Ontario						
Oncorhynchus kisutch	Su84	10				
Lake Nipigon						
Coregonus artedii	Su83	52	21.9±1.3 (13.9-25.4)	21	28±9.4	74
Coregonus clupeaformis	Su83,W85	38	?	83	87	0
Prosopium cylindraceum	Su83	1	29.2	0	–	–

*W,December-March; S,April-May; Su,June-August: F,September-November.

**Mean fork length (cm) ± SE, subtended by range in parentheses.

+Mean ± SE.

++Does not include S84 and Su84.

larvae for experimental infections. Mortality was high (up to 100%) in both infected and uninfected amphipods. No natural *C. farionis* infections were found in any amphipods used in experiments. Amphipods were viewed with a stereomicroscope after they had been introduced into a dish of water containing eggs of *C. farionis*. Currents created by the movement of the appendages and swimming motions of the amphipods caused the eggs to circulate throughout the water. Eggs readily adhered to appendages where setae were present. Scanning electron microscopy revealed that filaments on the eggs had wrapped around the setae and sometimes adhered to flat surfaces on the amphipod (Figs. 2-5). Most eggs adhered in areas where there were two or more adjacent setae for attachment. Live amphipods frequently groomed their antennae, clearing off eggs and debris with their gnathopods.

Success in infecting amphipods was dependent on the duration of egg storage (Table 5). *Cystidicola farionis* eggs stored at 6°C for 2 to 17 weeks in dechlorinated water, distilled water or saline did not hatch in the gut of amphipods. Eggs were still present in the gut up to 24 hours after exposure. No larvae were found in the haemocoel of any amphipod that was exposed to eggs stored for more than 2 weeks. Only eggs recently removed (<48 hours) from live worms hatched when fed to amphipods (Table 5). Most hatched within 24 hours postexposure (PE). First-stage larvae were found in the haemocoel as early as 4 hours PE.

Large batches of amphipods were exposed to eggs recently removed from live *C. farionis*. Six hundred *H. azteca* and 1,500 *G. fasciatus* were exposed to eggs from worms in lake whitefish from Pettit Lake. Eight hundred *H. azteca* and 1,500 *G. fasciatus* were exposed to eggs from worms in rainbow trout and lake herring respectively from Lake Superior. Mortality of amphipods was high (83% to 97%) in all four groups after 5 to 8 weeks. Only one third-stage larva was recovered from

Figures 2-5. Scanning electron micrographs of an amphipod (*Gammarus* sp.) after exposure to eggs of *Cystidicola farionis*. Fig. 2. Anterior end of amphipod. *ant*, second antenna; *g*, gnathopod. Bar=0.5 mm. Fig. 3. Second gnathopod with five eggs attached. Bar=50 μ. Fig. 4. Second antenna with three eggs attached. Bar=50 μ. Fig. 5. Attachment of filaments to setae and flat surface of second antenna. Bar= 20 μ.

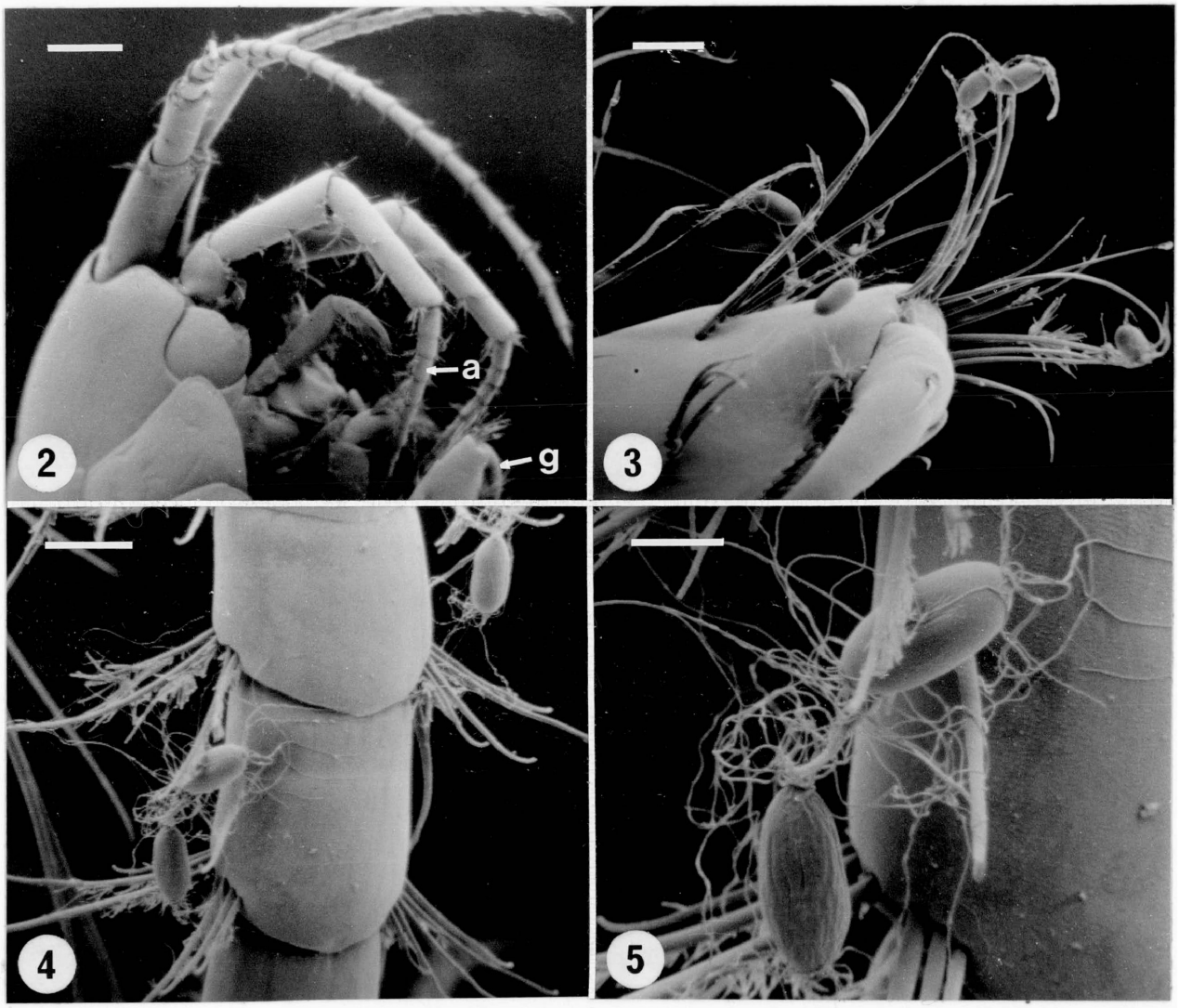

a
g
2
3
4
5

Table 5. Attempts to infect *Hyalella azteca* with *Cystidicola farionis* from various sources.

Source of *C. farionis* eggs	Storage medium	Period of egg storage	Exposure period (hours)	Examined after	No. of amphipods with eggs in gut	No. of amphipods with larvae in gut or haemocoel
Salmo gairdneri, Lake Superior	dechlorinated water	5 weeks	10	5 weeks	—*	0/4**
Coregonus clupeaformis, Greenwater Lake	distilled water	4 weeks	?	2 weeks	—*	0/10
Salmo gairdneri, Lake Superior	distilled water	12 weeks	-	3 days	—*	0/10
Coregonus clupeaformis, Lac Des Mille Lacs	distilled water	3 weeks	18	3 hours 24 hours	—* —*	0/10 0/10
Salmo gairdneri, Lake Superior	distilled water	15 weeks	?	1 hour 24 hours	2/5** 2/5	0/5 0/5
Salvelinus namaycush, Kluane Lake	saline	2 weeks	-	3 hours 24 hours	2/3 2/5	0/3 0/5
Coregonus clupeaformis, Greenwater Lake	distilled water	12 weeks	?	1 hour 10 days 3 weeks	1/2 0/3 0/16	0/2 0/3 0/16
Salmo gairdneri, Lake Superior	dechlorinated water	17 weeks	-	2 hours	2/20	0/20
Coregonus artedii, Lake Superior	saline	<48 hours	3.5	4 hours 24 hours 48 hours	3/4 0/6 0/5	3/4 5/6 3/5
Coregonus artedii,+ Lake Superior	saline	<48 hours	18	24 hours	7/10	10/10

*Lumen of gut not examined for eggs.

**No. of amphipods containing eggs or larvae/No. of amphipods examined.

+*Gammarus fasciatus* rather than *Hyalella azteca* exposed to eggs.

one of five *H. azteca* examined 8 weeks PE to eggs of *C. farionis* in lake whitefish from Pettit Lake. No larvae were found in five amphipods from each of the three remaining groups when examined 5 to 6 weeks PE.

Experimental infection of fishes

Success in infecting fishes by feeding exposed amphipods was poor. Only one worm was recovered in four attempts. Five *H. azteca*, exposed 8 weeks earlier to eggs from worms in lake whitefish from Pettit Lake, were fed to each of 10 rainbow trout and 10 lake whitefish. Only one fourth-stage larva (7.2 mm long) of *C. farionis* was recovered from one lake whitefish examined at 70 days PE. None of the remaining fishes, examined at 35, 70, 90, 105, and 150 days PE, was infected. Eleven *H. azteca*, exposed 6 weeks earlier to eggs from worms in rainbow trout from Lake Superior, were fed to each of two lake whitefish and two rainbow trout. No worms were recovered at 50 days PE. One rainbow trout and one lake whitefish, each given 20 *G. fasciatus* exposed 5 weeks earlier to eggs from worms in lake whitefish from Pettit lake, were not infected when examined at 30 days PE. Similarly, no worms were recovered after 30 days from one rainbow trout and one lake whitefish each given 20 *G. fasciatus* exposed 5 weeks earlier to eggs from worms in lake herring from Lake Superior.

Attempts were made to infect lake whitefish and rainbow trout with immature *C. farionis* from the swimbladder of lake trout and rainbow trout of Lake Superior. Up to 30 larvae (stage of development unknown) were stomach-tubed into lake whitefish and rainbow trout but no worms were recovered 48 hours later. Fish commonly regurgitated. This may be due to a side effect of the anesthesia which was necessary for stomach-tubing (see Black 1980). Up to 40 larvae were fed to rainbow trout in gelatin capsules, but no infections resulted.

A trial experiment showed that fish would readily consume a section of an

earthworm into which larvae had been injected. After 31 hours, one worm was recovered in the swimbladder, two in the esophagus and two in the stomach (one of which was dead) of a lake whitefish given 50 larvae in this way. Using the earthworm technique, 10 rainbow trout and nine lake whitefish, were each given 100 *C. farionis* larvae from Lake Nipigon lake whitefish. Examination of a subsample of larvae used for infections revealed that 94% (49 of 52) were still in the third stage; the rest were fourth-stage.

Eight of 10 of the exposed rainbow trout died 24 to 48 hours later of causes unrelated to the experimental infection. Four of these fish had from 1 to 18 worms ($\bar{x}$=9) that were 3.5 to 7.5 mm long ($\bar{x}$=5.6 mm) (Table 6). No worms were found in one rainbow trout examined at 90 days PE. Two fifth-stage, non-gravid female worms, 11.2 and 11.9 mm long, and one fourth-stage female, 10.5 mm long, were found in the last rainbow trout examined at 120 days PE. Temperature fluctuated from 8 to 12°C over the 120 days.

Lake whitefish were examined at 30, 60, 77, 90, 120 and 144 days PE (Table 7). Temperature ranged from 12 to 17°C for the first 80 days and from 8 to 12°C for the duration of the experiment. *Cystidicola farionis* from Lake Nipigon lake whitefish grew rapidly in hatchery-reared lake whitefish (Table 8, Fig. 6). Five of six worms had undergone the third moult by 30 days PE. Only fourth- and fifth-stage worms were recovered after 60 days. A fifth-stage male worm (9.9 mm long) with sclerotized spicules, caudal papillae and a tail beginning to coil, was recovered at 60 days PE. A mature male worm (11.5 mm long) was found at 77 days PE. One female worm (11.3 mm long) was undergoing the fourth moult at 77 days PE. Fifth-stage female worms (15.6-21.5 mm long) with unshelled ova in their uteri, were found at 120 days and 144 days PE (Table 8). Eleven of 19 fish (59%) were successfully infected using the earthworm technique. Less than five percent

Table 6. Recovery of *Cystidicola farionis* from experimentally infected rainbow trout (*Salmo gairdneri*)*.

	Days after exposure		
	2	90	120
No. of fish examined	8	1	1
No. of fish infected	4	0	1
Intensity	9(1-18)+	—	3
Third-stage larvae	33	-	0
Fourth-stage larvae	3	—	1
Fifth-stage worms	0	—	2

*Hatchery-reared rainbow trout (26-32 cm), were each given 100 *C. farionis* larvae from Lake Nipigon lake whitefish and held at 8-12°C.

+Mean intensity followed by range in parentheses.

Table 7. Recovery of *Cystidicola farionis* from experimentally infected lake whitefish (*Coregonus clupeaformis*)*.

	Days after exposure					
	30	60	77	90	120	144
No. of fish examined	2	2	1	1	1	2
No. of fish infected	1	1	1	0	1	1
Intensity	6	4	15	—	2	2
Third-stage larvae	1	0	0		0	0
Fourth-stage larvae	5	2	9		0	0
Fifth-stage worms	0	2	6	—	2	2

*Hatchery-reared lake whitefish (17-21 cm), were each given 100 *C. farionis* larvae from Lake Nipigon lake whitefish and held at 8-17°C.

Table 8. Major dimensions (in micrometres, unless otherwise mentioned) of developing *Cystidicola farionis* from wild and from experimentally infected lake whitefish (*Coregonus clupeaformis*)*.

	Third-stage larvae from Lake Nipigon lake whitefish		Developing *C. farionis* from lake whitefish, 2-150 days postinfection					
			Third-stage larvae		Fourth-stage larvae		Fifth-stage worms	
Sex	♂	♀	♂	♀	♂	♀	♂	♀
n	5	5	1	0	5	11	7	5
Length, mm	5.3(4.1-6.4)**	5.4(4.1-6.8)	5.7	–	7.5(6.6-8.1)	10.5(7.4-12.4)	10.1(9.0-11.5)	16.6(11.3-21.5)
Buccal cavity	82(75-92)	82(74-88)	90		96(90-102)	107(98-112)	113(104-125)	125(112-141)
Nerve ring+	174(155-197)	175(154-200)	197		218(202-230)	242(218-256)	244(225-265)	297(267-310)
Width at nerve ring	53(46-56)	56(50-61)	52		62(52-70)	78(61-95)	78(72-90)	94(70-105)
Excretory pore	263(235-296)	260(218-293)	305		332(310-360)	369(320-435)	372(345-420)	437(385-475)
Esophagus								
Glandular, mm	0.92(0.78-1.04)	0.91(0.69-1.13)	1.00		1.15(0.95-1.30)	1.54(1.20-1.85)	1.53(1.35-1.85)	1.99(1.40-2.40)
Muscular	246(205-285)	249(210-290)	280		285(252-330)	349(310-387)	343(309-405)	419(342-455)
Total, mm	1.17(0.99-1.33)	1.16(0.90-1.43)	1.28		1.44(1.21-1.66)	1.89(1.53-2.24)	1.87(1.66-2.26)	2.41(1.74-2.86)
% of body length	23(20-27)	22(20-26)	16		19(17-23)	18(16-21)	19(16-20)	15(13-16)
Oral opening++	15	15	16		23(22-25)	26(23-27)	31(26-36)	40§
Width at anus	45(40-53)	41(35-48)	39		53(45-65)	53(38-65)	78(70-92)	70(55-82)
Tail length	105(83-128)	87(70-115)	103		120(108-135)	95(75-112)	178(155-200)	139(95-172)
Vulva, mm+	–	3.1(2.4-3.8)	–		–	6.2(5.1-7.5)	–	8.5(7.4-11.0)
Left spicule	–	–	–		–	–	719(550-890)	–
Right spicule	–	–	–	–	–	–	160(135-185)	–

*Hatchery-reared lake whitefish of Lake Simcoe stock, were each given 100 *C. farionis* larvae from Lake Nipigon lake whitefish and held at 8-17°C.

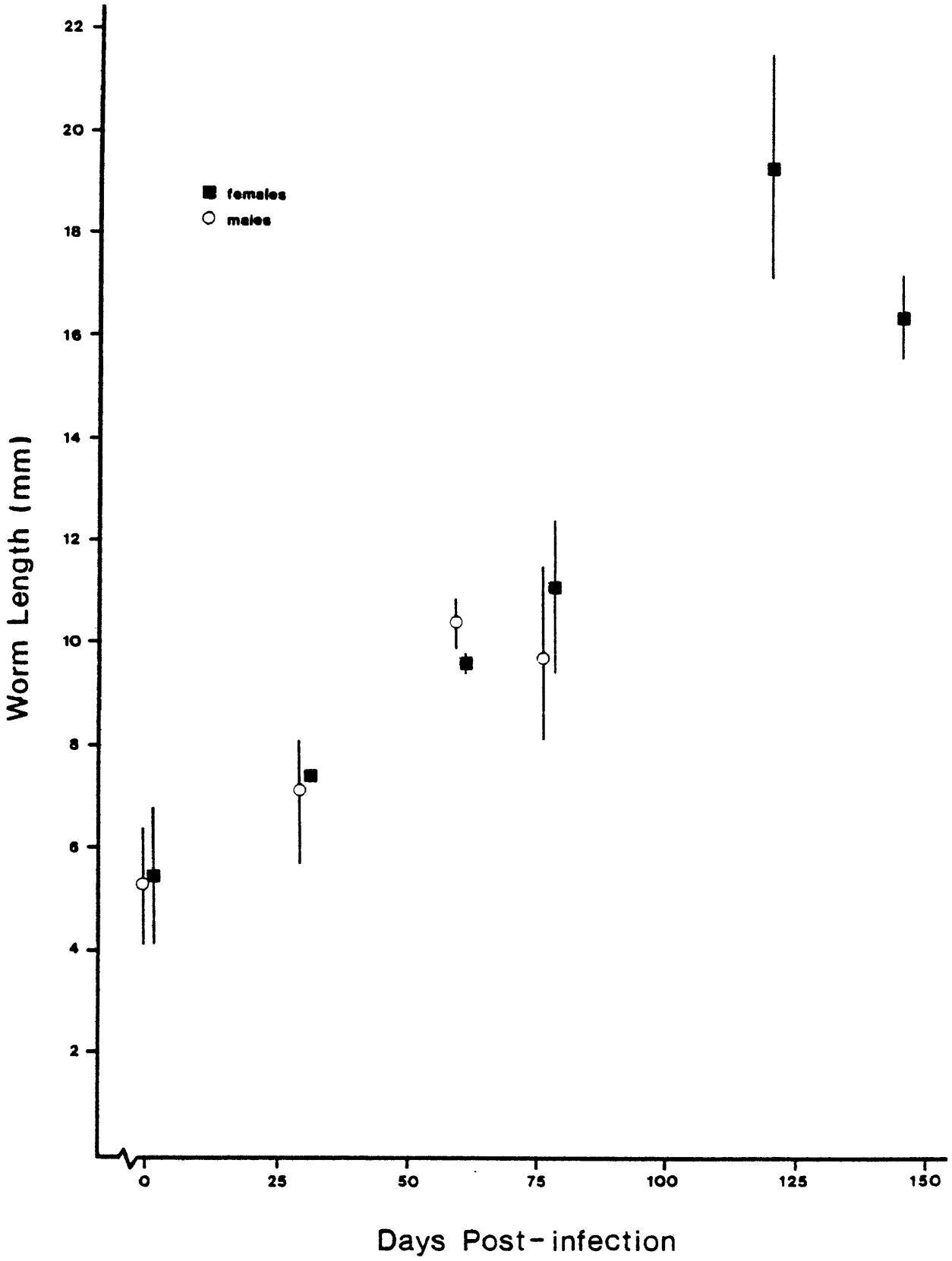
**Mean followed by range in parentheses.

+Distance from structure to anterior end.

++Dorsal-ventral dimension.

§n=1.

Figure 6. Growth of *Cystidicola farionis* from Lake Nipigon lake whitefish in experimentally infected lake whitefish, *Coregonus clupeaformis*, of Lake Simcoe origin, held at 8-17°C. Points represent means, bars represent ranges.


22
20
18
16
14
12
10
8
6
4
2
females
males
Worm Length (mm)
0
25
50
75
100
125
150
Days Post-infection

of the larvae originally given to fish were recovered.

Since rainbow smelt from Lake Superior are commonly infected with immature *C. farionis*, their possible role in the transmission of swimbladder nematodes to piscivorous salmonids was investigated. *Cystidicola farionis* was recovered from 75% of the rainbow smelt examined. Infected rainbow smelt had from one to eight worms ($\bar{x}$=4). Eighteen of 62 worms recovered had not developed beyond the third stage (Table 9). There was considerable overlap in length of larvae in successive developmental stages. The dorso-ventral dimension of the oral opening proved useful in distinguishing larvae in different stages (Table 9). No overlap existed between stages and the measurement was readily made on worms viewed laterally from whole mounts or from *en face* preparations. The rainbow trout died 148 days after being fed 20 rainbow smelt and no worms were present in the swimbladder.

Morphology of Cystidicola *spp.*

The morphology of adult *C. farionis* in lake whitefish from inland lakes, and those in rainbow trout and other salmonids from Lake Superior was compared. No significant morphometric differences were detected in adult worms (Table 10). However, the number and distribution of filaments on eggs differed between hosts (Table 11, Fig. 7). Eggs from worms in lake whitefish had more lateral filaments and a greater ratio of lateral to polar filaments (L/P) than eggs from worms in rainbow trout and other salmonids (lateral filaments, $F_{5,167}$=37.1; L/P, $F_{5,167}$=94.3). Polar filament counts did not differ between eggs of worms in lake whitefish, lake herring and rainbow trout (Table 11). However, eggs of worms in coho salmon had more polar filaments than eggs from worms in these three hosts and pink salmon

Table 9. Dimensions and stage of development of *Cystidicola farionis* from 20 rainbow smelt (*Osmerus mordax*) collected from McVicar Creek, Thunder Bay, Ontario, in April 1984.*

	Stage of development		
	Third-stage	Fourth-stage	Fifth-stage
n			
total	18	39	5
males	8	17	4
females	10	22	1
% of total	29	63	8
length, mm			
males	6.0±0.24** (5.1-7.2)	8.3±0.35 (6.7-11.2)	11.9±0.65 (10.7-12.9)
females	5.9±0.32 (3.7-7.4)	9.6±0.50 (7.2-14.5)	15.2
Oral opening, μm+	14.9±0.26 (13.5-16.5)	22.1±0.24 (19.5-27.0)	32.0±1.32 (30.0-34.5)

*Fork length 12.1-15.8 cm ($\bar{x}$=13.7±0.23), prevalence=75 %, mean intensity=4.1.

**Mean ± SE, subtended by range in parentheses.

+Dorsal-ventral dimension of opening.

Table 10. Major dimensions (in micrometres, unless otherwise mentioned) of 25 male and 25 female *Cystidicola farionis* from Greenwater Lake lake whitefish (*Coregonus clupeaformis*) and Lake Superior rainbow trout (*Salmo gairdneri*).

	Host			
	Coregonus clupeaformis		*Salmo gairdneri*	
Sex	♀	♂	♀	♂
Length, mm	24.8±4.89* (15.2-34.5)	18.6±4.17 (12.6-28.8)	29.5±5.77 (18.7-39.9)	21.2±3.35 (12.9-26.1)
Buccal cavity**	156±14.9 (130-180)	141±10.8 (125-170)	150±16.3 (110-175)	143±17.9 (110-175)
Esophagus				
Glandular, mm	2.80±0.49 (1.40-3.80)	2.41±0.44 (1.60-3.25)	2.84±0.38 (1.95-3.40)	2.60±0.38 (1.60-3.25)
Muscular	485±44.6 (405-580)	435±61.2 (340-550)	510±61.2 (410-640)	496±49.5 (420-580)
Total, mm	3.28±0.52 (1.84-4.38)	2.84±0.49 (1.94-3.80)	3.35±0.42 (2.41-4.04)	3.10±0.41 (2.03-3.76)
% of body length	13.4±1.55 (10.7-17.1)	15.5±1.48 (11.8-17.8)	11.6±1.77 (8.87-17.0)	14.7±1.26 (12.9-17.4)
Nerve ring**	365±36.1 (300-420)	323±36.9 (265-425)	354±32.3 (305-420)	343±34.9 (250-400)
Width at nerve ring	129±12.6 (105-145)	107±12.8 (90-140)	115±9.1 (95-130)	101±11.6 (75-120)
Excretory pore**	573±79.2+ (420-730)	519±57.6+ (410-650)	575±63.3 (470-710)	555±62.9+ (420-670)
Width at anus	103±17.1 (85-145)	86±11.7 (65-115)	102±18.6 (70-160)	94±10.3 (75-115)
Tail length	179±36.8 (120-270)	251±33.7 (180-320)	205±27.7 (160-280)	295±47.2 (175-365)
Left spicule	—	814±66.6 (695-985)	—	865±63.7 (755-1000)
Right spicule	—	194±19.7 (160-240)	—	203±15.4 (170-240)
Ratio of left spicule to right spicule	—	4.22±0.49 (3.54-5.56)	—	4.27±0.32 (3.69-4.95)
Vulva, mm	12.9±2.59++ (8.5-18.8)	—	14.7±2.84 (9.5-21.0)	—
% of body length	53.2±3.09++ (48.6-59.2)	—	50.0±3.45 (42.2-55.0)	—
Eggs				
Length	48±0.3 (46-52)	—	47±0.2 (45-49)	—
Width	27±0.2 (26-29)	—	27±0.4 (25-29)	—

*Mean ± SD, subtended by range in parentheses.

**Distance from structure to anterior end.

+n=24.

++n=22.

Table 11. Arrangement and numbers of filaments on 30 eggs of *Cystidicola farionis* from wild fishes.

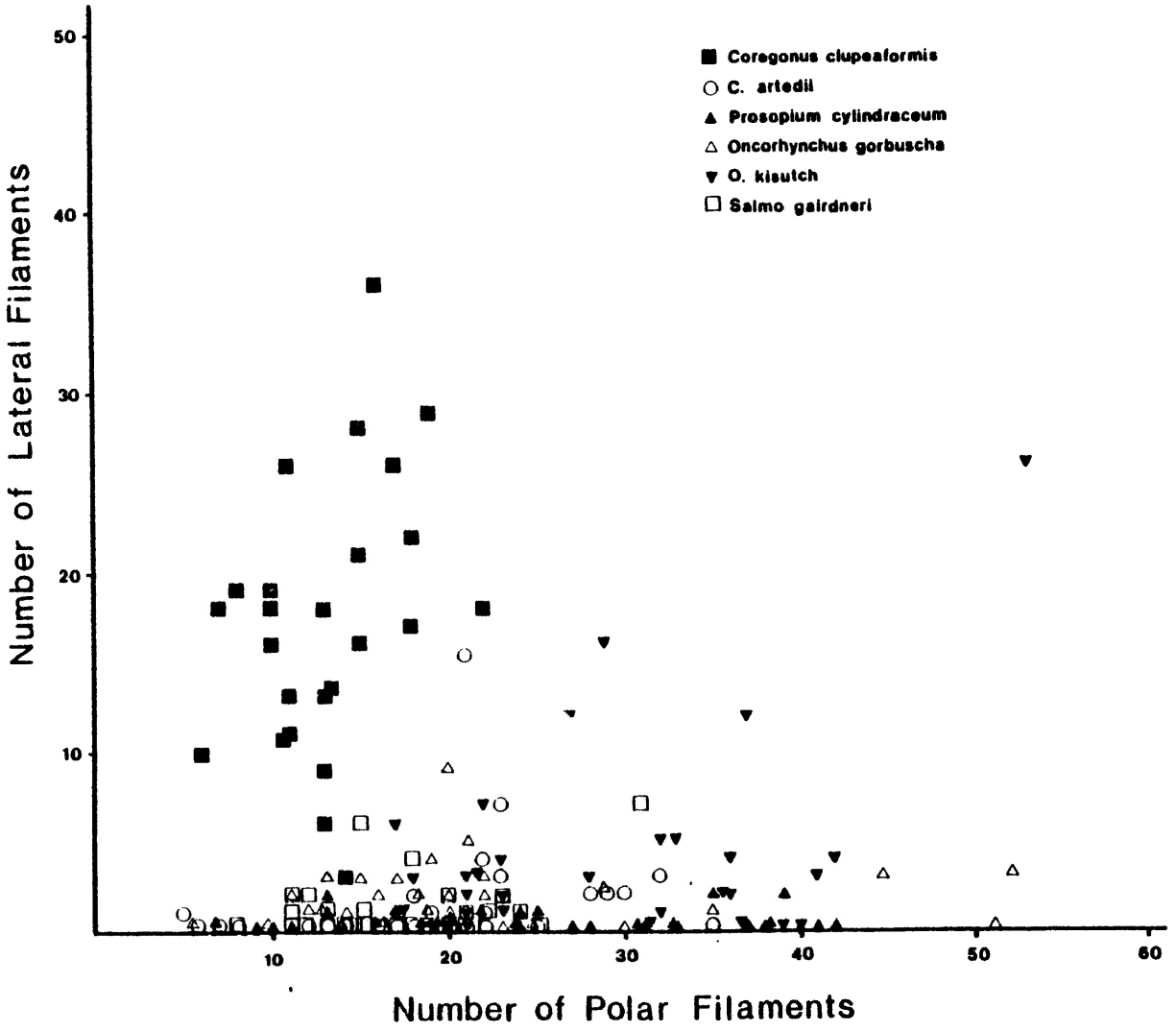
Host species and location	% with polar filaments	% with lateral filaments	% with polar and lateral filaments	Number of polar filaments*	Number of lateral filaments*	Ratio of lateral to polar filaments*
Coregonus artedii						
Lake Nipigon	100	7	7	–	–	–
Lake Superior	100	50	50	19.7±1.39^{abc} ** (5-35)	2.9±1.41^{a} (0-40)	0.13±0.06^{a} (0-1.54)
Coregonus clupeaformis						
Lake Superior	73	100	73			
Lake Ontario	77	100	77			
Lake Huron	63	100	63			
Greenwater Lake, Ont.	100	97	97	–	–	–
Pettit Lake, Ont.+	100	100	100	13.2±0.77^{a} (6-22)	17.4±1.52^{b} (3-36)	1.39±0.12^{b} (0.21-2.57)
Cold Lake, Alta.	100	80	80	–	–	–
Slave River, YT	100	97	97		–	–
Prosopium cylindraceum						
Lake Superior	100	30	30	25.4±2.05^{cd} (7-47)	1.1±0.72^{a} (0-22)	0.03±0.02^{a} (0-0.47)
Oncorhynchus gorbuscha						
Lake Superior	100	80	80	21.5±2.03^{bc} (6-52)	1.9±0.34^{a} (0-9)	0.10±0.02^{a} (0-0.45)
Oncorhynchus kisutch						
Lake Superior	100	80	80	29.2±1.66^{d} (17-53)	4.2±1.03^{a} (0-26)	0.14±0.03^{a} (0-0.50)
Salmo gairdneri						
Lake Huron	100	30	30			
Lake Superior	100	27	27	17.2±0.94^{ab} (8-31)	1.0±0.32^{a} (0-7)	0.06±0.02^{a} (0-0.48)
Salvelinus alpinus						
Willow Lake, NWT	100					
Salvelinus namaycush						
Pettit Lake, Ont.	93	90	83			
Simmons Lake, BC	100	0	0		–	
Aishihik Lake, YT	100	3	3	–	–	–

*Determined only for eggs viewed with scanning electron microscope.

**Mean ± SE, subtended by range in parentheses. Means followed by the same letter are not significantly different ($p \geq 0.05$).

+n=25.

Figure 7. Distribution and numbers of filaments on eggs of *Cystidicola farionis* in lake whitefish, *Coregonus clupeaformis*, from Pettit Lake, Ont. and in salmonids from Lake Superior.


Coregonus clupeaformis
C. artedii
Prosopium cylindraceum
Oncorhynchus gorbuscha
O. kisutch
Salmo gairdneri
Number of Lateral Filaments
Number of Polar Filaments
10
20
30
40
50
60

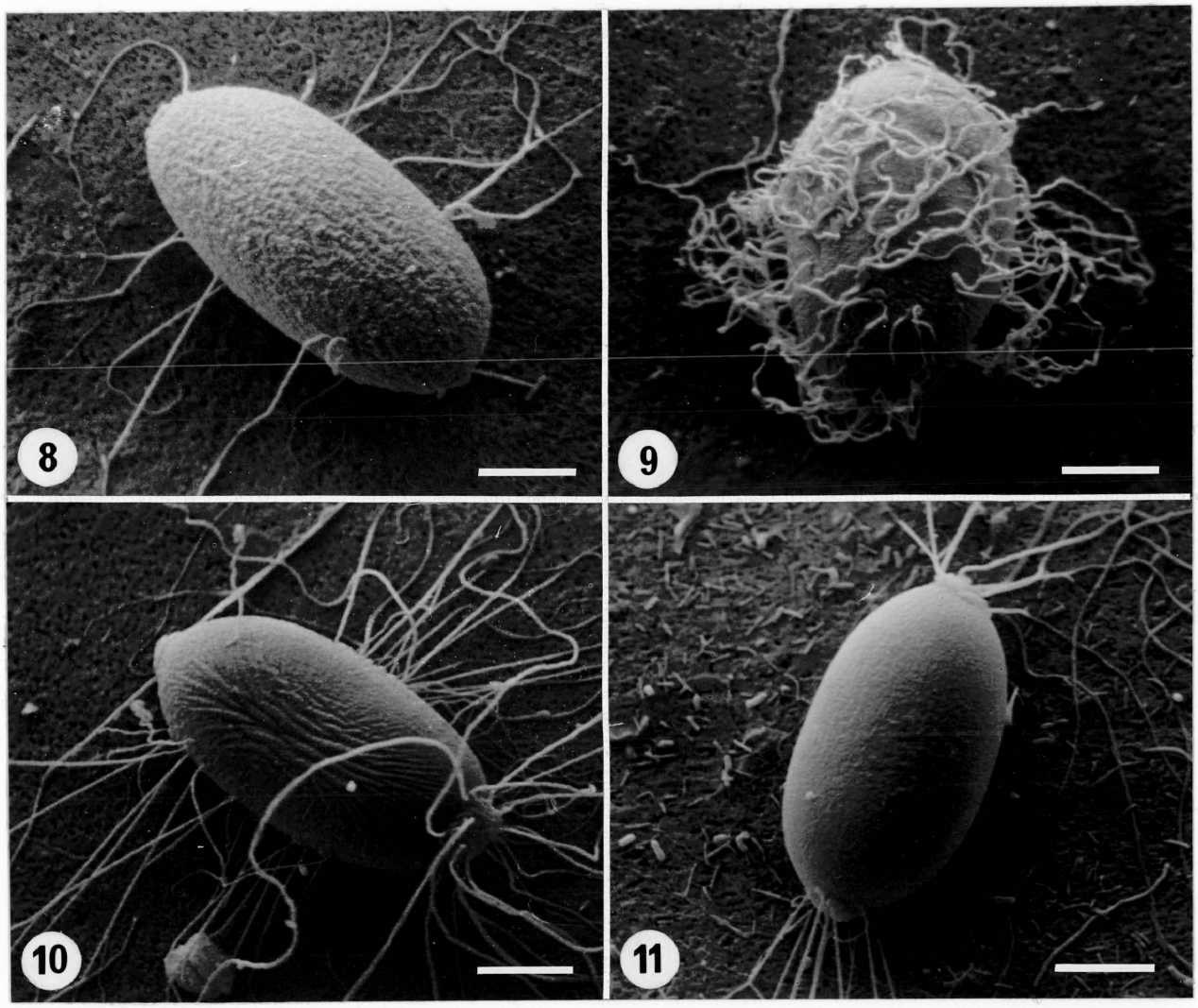
($F_{5,167}$=12.7). The number of polar filaments on eggs of worms in pink salmon and round whitefish also differed from numbers of polar filaments on eggs of worms in some of the other hosts examined (Table 11). Most eggs from worms in lake whitefish had lateral filaments originating from opposing lateral fields (Fig. 8), but some had filaments originating anywhere on the lateral surface (Fig. 9). It was difficult to make accurate filament counts on such eggs and the actual numbers of lateral filaments are probably higher than recorded. Ninety-six percent of *C. farionis* eggs from lake whitefish had lateral filaments; 87% had polar filaments (overall means). In contrast, only 21% of nematode eggs from rainbow trout and lake herring had lateral filaments (Fig. 10), while all eggs had polar filaments (Fig.11). Only polar filaments existed on eggs from worms in lake trout from western Canada. Ninety percent of *C. farionis* eggs from lake trout from Pettit Lake had lateral filaments. These eggs appeared similar to those in lake whitefish from the same lake. Polar and lateral filaments on *C. farionis* eggs from rainbow trout (polar, $\bar{x}$=131μ ±4.2; lateral, $\bar{x}$=83μ ±2.9) were longer than those on eggs from lake whitefish (polar, $\bar{x}$=51μ±2.2; lateral, $\bar{x}$=46μ±2.2) ($F_{3,117}$=170.4).

Examination with the scanning electron microscope allows for a more detailed description of the cephalic and cervical structures as well as the eggs of *C. farionis* and its close relative *C. stigmatura* than heretofore available.

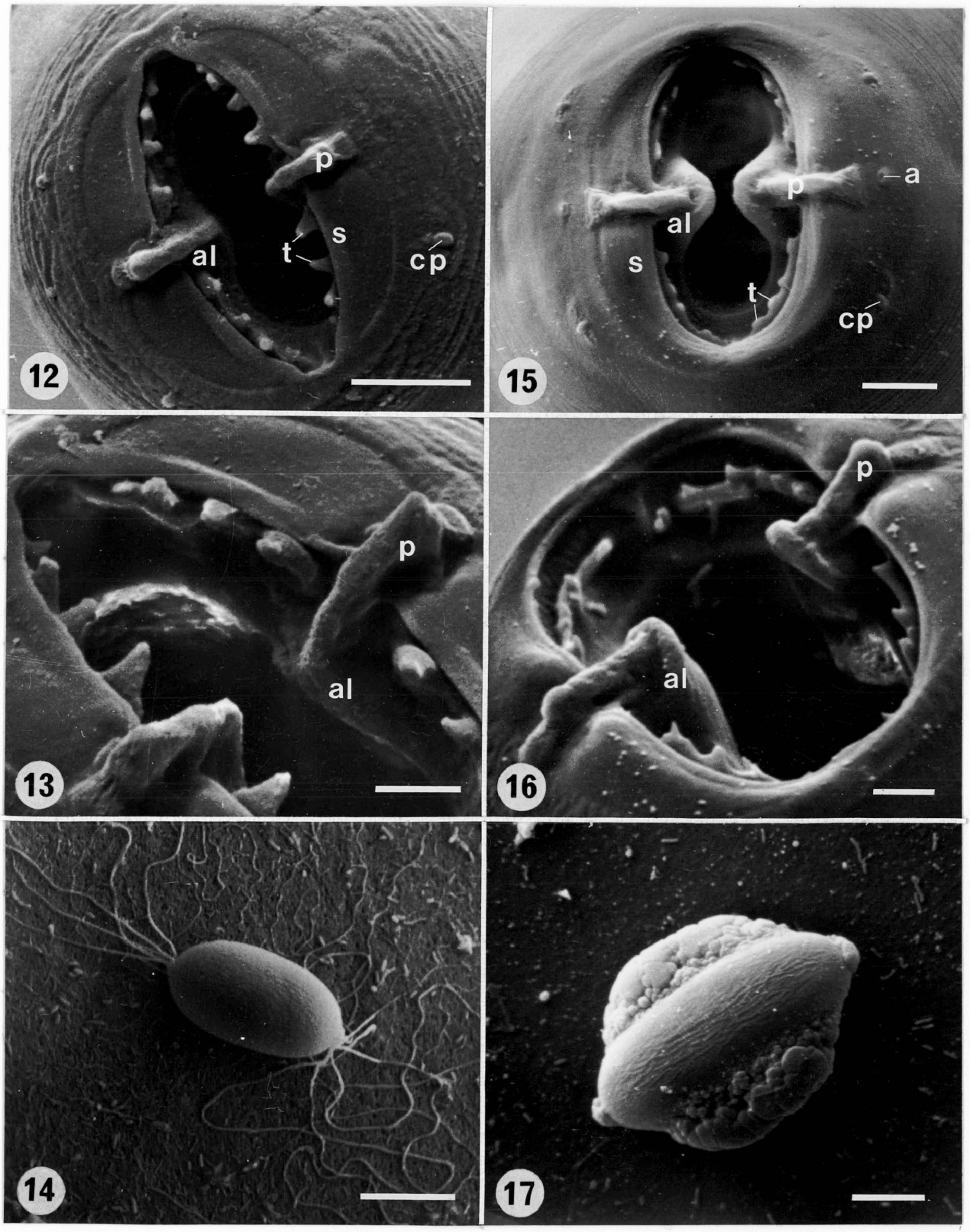
Cystidicola farionis (Figs. 12-14,18,19)

Oral opening, dorso-ventrally elongate. Four cephalic papillae. Amphids lateral at base of pseudolabia. Pseudolabium with conical apex; narrow in lateral view. Submedian labia forming dorsolateral and ventrolateral margins of oral opening. Four sublabial teeth in each subdorsal and subventral quadrant of oral

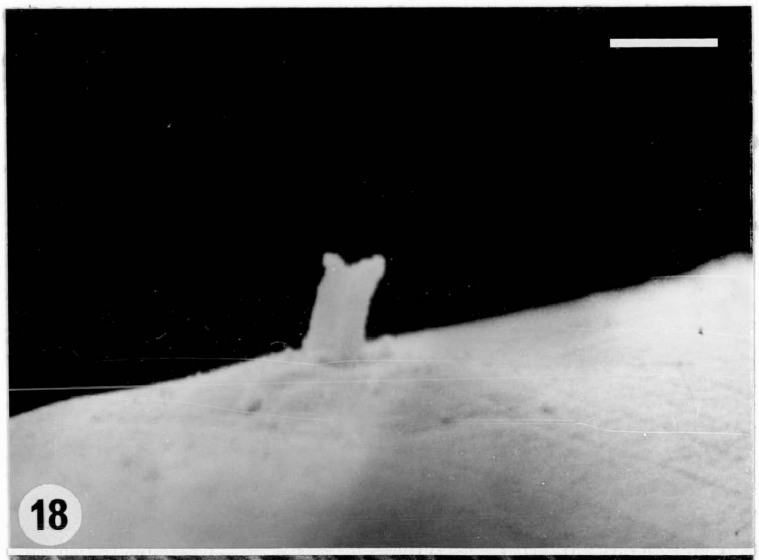
Figures 8-11. Scanning electron micrographs showing variation in the morphology of *Cystidicola farionis* eggs. Bar= 10 μ . Fig. 8. Egg with lateral filaments arising from opposite fields; from worm in lake whitefish, *Coregonus clupeaformis*, from Pettit Lake, Ont. Fig. 9. Egg with lateral filaments arising from entire lateral surface; from worm in lake whitefish from Pettit Lake, Ont. Fig. 10. Egg with polar and lateral filaments from worm in lake herring, *Coregonus artedii*, from Lake Superior. Fig. 11. Egg with polar filaments from worm in rainbow trout, *Salmo gairdneri*, from Lake Superior.


8
9
10
11

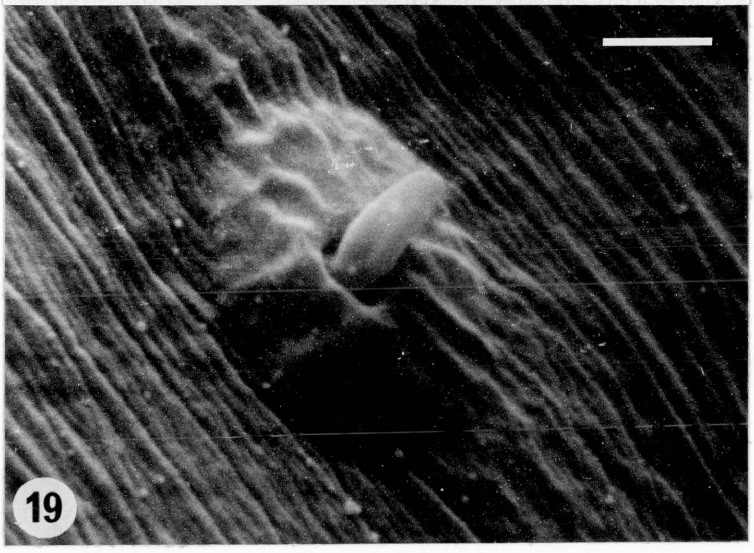
Figures 12-17. Scanning electron micrographs of *Cystidicola* spp. *a*, amphid; *al*, anterolateral extension of buccal cavity wall; *cp*, cervical papilla; *p*, pseudolabium; *s*, submedian labium; *t*, sublabial teeth. Fig. 12. *En face* view of *C. farionis* in coho salmon, *Oncorhynchus kisutch.* Bar=10 μ . Fig. 13. Posterolateral view of cephalic extremity. Bar=4 μ . Fig. 14. Egg of *C. farionis* with polar filaments from rainbow trout, *Salmo gairdneri.* Bar=20 μ . Fig. 15. *En face* view of *C. stigmatura* in lake trout, *Salvelinus namaycush.* Bar=10 μ . Fig. 16. Dorsoventral view of cephalic extremity of *C. stigmatura* in lake trout. Bar=4 μ . Fig. 17. Egg with opposite lateral lobes from *C. stigmatura* in lake trout. Bar=10 μ.


12
p
s
t
al
cp
15
a
p
al
s
t
cp
13
p
al
16
p
al
14
17

Figures 18-19. Scanning electron micrographs of deirids of *Cystidicola farionis* Bar=2 μ. Fig 18. Bifurcate deirid of *C. farionis* in lake whitefish, *Coregonus clupeaformis.* Fig. 19. Deirid of *C. farionis* from rainbow trout, *Salmo gairdneri.*



18



19

opening. Sublabial teeth acute to bluntly rounded. Anterolateral wall of buccal cavity without a lip-like projection fused to medial aspect of pseudolabium. Deirids small, located just anterior to nerve ring; entire or bifurcate distally with a longitudinal groove in the sagittal plane. Eggs with polar and/or lateral filaments; up to twice the length of the egg.

There were no morphological differences detected between *en face* preparations or spicules of worms from lake whitefish and rainbow trout viewed with the SEM. The deirids of three worms from lake whitefish were bifurcate distally (Fig. 18); the rest appeared entire (20 worms examined). All deirids of *C. farionis* from rainbow trout appeared entire distally (Fig. 19) (20 worms examined).

Cystidicola stigmatura (Figs. 15-17)

The cephalic extremity of *C. stigmatura* is similar to that of *C. farionis* and only differences are described. Usually four sublabial teeth in each subdorsal and subventral quadrant of oral opening. Sublabial teeth usually bluntly rounded. Anterolateral wall of buccal cavity with a prominent lip-like projection fused to medial aspect of pseudolabium. Deirids small, located just anterior to nerve ring; bifurcate distally with a longitudinal groove in the sagittal plane (four specimens examined). Eggs with opposite lateral lobes.

Cystidicola stigmatura is easily distinguished from *C. farionis* by the presence of lobes rather than filaments on mature eggs and the prominent lip-like projection of the anterolateral wall of the buccal cavity. Also, the uterus of *C. farionis* is of larger diameter, more highly convoluted, and more completely fills the pseudocoel, than that of *C. stigmatura.* Up to 40 times the number of eggs are present in the uteri of *C. farionis* than in similar sized *C. stigmatura* (Table 12).

Table 12. Estimated numbers of eggs in female *Cystidicola* spp.

Worm length (mm)	*C. farionis**	*C. stigmatura*+
27	–	487
28	21344	–
32	–	1380
33	32560	1460
	–	1853
34	38280	–
35	41269	–
37	55152	3577

*Worms in *Salmo gairdneri* from Lake Superior.

+Worms in *Salvelinus namaycush* from Icarus Lake.

Discussion

No morphological differences were detected between adult worms in rainbow trout and lake whitefish. For all characters measured, broad overlap occurs between host species. Scanning electron microscopy of the cephalic extremity of *C. farionis* in lake whitefish and rainbow trout did not reveal any morphological differences in the worm between hosts. Similarly, Ko and Anderson (1969) found that swimbladder nematodes in lake whitefish from Lake Huron (then *C. stigmatura*) and those in Arctic charr from English waters (*C. farionis*) were morphologically indistinguishable. It is not known if the variation in deirid morphology seen within *C. farionis* is real or an artifact of preparation. The cuticle around deirids was often distorted due to the high magnifications and high voltages required to view detail. The small size and location of deirids on the worm also made viewing difficult.

Scanning and light microscopy revealed that eggs of worms in lake whitefish have a predominance of lateral filaments while those in all other salmonid hosts have mainly polar filaments. A notable exception is worms in lake trout from Pettit Lake, which had lateral filaments on 90% of their eggs. These eggs are similar to those from worms in lake whitefish from the same lake. Polar filaments predominated on eggs of *C. farionis* in lake trout from lakes in northwestern Canada. Ko and Anderson (1969) cautioned that filaments on the eggs of *Cystidicola* spp. are of little taxonomic value due to the high degree of variability which exists within host species. Despite the variability within host species, differences in the numbers of polar and lateral filaments can be detected between host species. Therefore the number and arrangement of filaments is useful in

distinguishing worms from different hosts.

Differences in filament arrangement on eggs might reflect genetic or species differences between worms in different hosts or they may be host-induced. Examination of *C. farionis* eggs from wild fishes provides evidence supporting both of these possibilities. Differences in egg morphology of worms in lake trout from northwestern Canada and those in lake trout from Pettit Lake suggest that host species does not affect filament arrangement. On the other hand, the arrangement of filaments on eggs of worms in salmonids from Lake Superior suggests that egg morphology is affected by species of host. Presumably, the introduced salmonids (rainbow trout, pink salmon and coho salmon) first acquired *C. farionis* infections from native fishes (lake herring and round whitefish). Numbers of polar filaments differ between these hosts implying host effects on egg morphology. Most swimbladder nematodes in lake whitefish from Lake Superior do not mature (Lankester and Smith 1980), but those that do have predominantly lateral filaments. Infections in this host may be acquired from other fishes in the lake, all of which have worms with eggs bearing mostly polar filaments. The possibility cannot be excluded, however, that lake whitefish with a race of swimbladder worm having lateral egg filaments exist in discrete areas of the lake or that such worms enter Lake Superior via the watershed and account for the small number of mature worms reported by Lankester and Smith (1980).

Swimbladder nematode eggs from coho salmon of Cold Lake, Alberta would provide a good test of possible host effects on egg morphology. When coho salmon were introduced into Cold Lake, they probably acquired *C. farionis* from lake whitefish (Leong 1975). *Cystidicola farionis* matures in coho salmon introduced into Lake Superior and the eggs have mainly polar filaments. These worms were probably acquired from lake herring and salmonids other than lake whitefish. A

comparison of eggs of worms in coho salmon from these two lakes with eggs in hosts from which coho salmon originally acquired infections would reveal whether fish host affects egg morphology. Unfortunately the mature worms reported in coho salmon from Cold Lake by Leong and Holmes (1981) were unavailable for study. Worms in lake trout from Lake Nipigon similarly would serve to test host effects on egg morphology. Small numbers of mature worms have been reported in lake trout from Lake Nipigon (Lankester and Smith 1980). Mature worms in lake herring from the same lake have eggs with mainly polar filaments. If the eggs of the few worms maturing in lake trout have predominantly polar filaments like those in lake herring, it would support the hypothesis that egg morphology is not affected by fish host. Unfortunately, specimens of *C. farionis* from Lake Nipigon lake trout also were unavailable for study. In Pettit Lake, the few worms which mature in lake trout are probably acquired from lake whitefish. Eggs of worms from these lake trout appear similar to those in lake whitefish from the same lake which have predominantly lateral filaments.

Mature *C. farionis* have been reported in lake whitefish from several smaller inland lakes in Ontario (Ko and Anderson 1969; Lankester and Smith 1980; this study) and western Canada (see Margolis and Arthur 1979; Black 1983b). The author is not aware of any infected populations of lake whitefish from small inland lakes, including those studied here, in which *C. farionis* does not mature. Conversely, only a small percentage of worms reach sexual maturity in lake whitefish from Lake Superior and none mature in this host from Lake Nipigon (Lankester and Smith 1980). The few lake whitefish from Lake Superior in which worms matured were collected from Pine Bay and from the east side of the Black Bay Peninsula (Lankester, unpublished). Mature *C. farionis* have been reported in lake whitefish from the North Channel and South Bay of Lake Huron (Bangham 1955; Lankester

and Smith 1980) and from the Bay of Quinte in Lake Ontario (Moody 1981). Ko and Anderson (1969) found only immature worms in two infected lake whitefish from Lake Ontario, but did not specify the locality within the lake. Hunter and Bangham (1933) found *Cystidicola* sp. in lake whitefish from Lake Erie, but did not mention if the worms were mature. Amin (1977) examined lake whitefish from Lake Michigan but did not report *C. farionis* nor mention if swimbladders were examined.

Lake herring are commonly infected with large numbers of mature *C. farionis* in Lakes Superior and Nipigon (Warren 1952; Lankester and Smith 1980; this study). Similarly, lake herring in Lakes Ontario and Erie once harboured large numbers of worms which reached sexual maturity (Skinker 1931; Hunter and Bangham 1933). Bangham (1955) reported *C. farionis* in lake herring from Lake Huron, but did not mention if they were mature.

The distribution and biology of *C. farionis* in lake herring from small inland lakes is puzzling. No infected lake herring were found from three lakes in which lake whitefish were infected with *C. farionis*. This suggests that lake herring may not be a suitable host for the swimbladder nematode which matures in lake whitefish. However, the worm's absence may be related to the feeding habits of lake herring in these lakes. None of 446 lake herring examined from Southern Indian Lake, Manitoba by Watson and Dick (1979) contained swimbladder nematodes although lake whitefish from the same lake were infected. The absence of the worm was attributed by the authors (Watson and Dick 1979) to the low frequency of amphipods in the diet of lake herring (<3%) and the suggestion that they may differ from the species eaten by lake whitefish. Leong and Holmes (1981) found only a few immature *C. farionis* in 2.5% of the lake herring they examined from Cold Lake, Alberta where lake whitefish are commonly infected with mature worms. The opposite situation exists in Lakes Nipigon and Superior where worms mature in lake

herring and never or only rarely mature in lake whitefish (Lankester and Smith 1980). This possible difference in host specificity supports the view that swimbladder nematodes in lake whitefish from inland lakes are distinct from *C. farionis*. Dechtiar (1972) reported *C. farionis* in both lake herring and lake whitefish from Lake of the Woods, Ont., but did not mention if the worms were mature in either host. Anthony (1963) found large numbers of *C. farionis* (=*C. stigmatura*) in the swimbladders of lake herring from Green and Little Green lakes in Wisconsin. Intensity ranged from 75 to 200 worms, similar to values for this host in Lake Superior. Presumably, these worms were mature as lake herring were the only salmonid fish present. Mature worms have also been reported in lake herring from Lake of Bays, Ont (Ko and Anderson 1969).

The ability of *C. farionis* to mature in other fish hosts also varies with locality. According to Valtonen and Valtonen (1978) and Fagerholm (1982), the smelt (*Osmerus eperlanus* L.) harbours mature *C. farionis* in Bothnian Bay, Finland, while no mature female worms are found in whitefish (*Coregonus laveratus* L.). In Lake Superior, neither the closely related rainbow smelt nor the lake whitefish are suitable hosts for *C. farionis* (Lankester and Smith 1980). Worms acquired from brown trout, mature in rainbow trout introduced into the River Itchen, Wales (Poynton 1985). In Lake Superior, where *C. farionis* matures in rainbow trout, brown trout are apparently unsuitable for the development of the parasite (Lankester and Smith 1980). Brown trout (*Salmo trutta* L.) have been reported as definitive hosts of *C. farionis* in several British rivers (Awachie 1973; Kennedy 1974; Conneely and McCarthy 1984; Poynton 1985).

Data do not demonstrate clearly whether differences in the worm's ability to mature in different hosts are due to the genetics of the worm, the host, or both. Experimental cross-infections were conducted to determine if differences in egg

morphology and host specificity observed in the field are due to genetic differences between the worms which mature in different hosts or are a reflection of the environment provided by the host.

The development of *C. farionis* in experimentally infected lake whitefish suggests that the worm's ability to mature may depend upon host strain. Immature *C. farionis* accumulate in the swimbladder of lake whitefish from Lake Nipigon without maturing (Lankester and Smith 1980). These worms grew up to 23.0 mm long and matured when given to rainbow trout by Black and Lankester (1980). When third-stage *C. farionis* larvae were administered to hatchery-reared lake whitefish (Lake Simcoe stock) in the present study, all worms grew and developed towards maturity. By 77 days all worms had undergone the fourth moult and a mature male worm was found. Although no mature females were recovered, fish examined at 120 and 144 days after infection, had only female worms in their swimbladders. These worms had undergone their final moult and were as large as some gravid worms reported in wild lake whitefish (Lankester and Smith 1980) but the eggs in their uteri were unfertilized and therefore unshelled. Lankester and Smith (1980) erroneously reported that immature *C. farionis* in rainbow smelt from Lake Superior were 15-36 mm long. An examination of their raw data (lankester unpublished) revealed that this is actually the length range of mature female worms in rainbow smelt. Mature male and immature worms were 9-20 mm long and 4-17 mm long respectively. All (n=4) *C. farionis* recovered after 120 days from experimentally infected lake whitefish were greater than 15 mm long; larger than any of the immature worms in wild lake whitefish from Lake Nipigon (Lankester and Smith 1980; this study). Since the development of *C. farionis* may be affected by strain of host, it is unwise to infer taxonomic differences based upon the ability of the worm to mature in different host species.

Differences in the suitability of host stocks or strains could reflect genetic differentiation among lake whitefish populations or may be caused by the effects of the environment in which the fish are found. One environmental factor which might affect the physiology of the swimbladder is the depth distribution of lake whitefish. Pressure is known to affect the concentrations of different gases within the swimbladder (Jones and Marshall 1953). Ihssen and Tait (1974) demonstrated that lake trout which inhabited different depths in separate lakes differed genetically in the gas retention abilities of their swimbladders. The differentiation of lake whitefish into genetically discrete stocks between and within lakes has been well documented (Casselman et. al. 1981; Ihssen et. al. 1981) and the existence of morphological sub-species within one lake has even been suggested (Kirkpatrick and Selander 1979). To determine if the inability of *C. farionis* to develop in lake whitefish from Lake Nipigon is environmentally induced or due to the genetics of the fish, infected lake whitefish from Lake Nipigon could be captured and maintained in the laboratory. Serial killings would determine if *C. farionis* develops in these fish under laboratory conditions which were suitable for the development of the worm in Lake Simcoe lake whitefish.

Differences in egg morphology between hosts suggests that more than one species of worm might exist. This conclusion must be considered speculative without the experimental evidence that host species does not affect egg morphology. In the absence of this information, the lack of differences in the morphology of adult worms between hosts and the observed growth and development of all worms recovered from experimentally infected lake whitefish, lead the author to believe that worms which mature in lake whitefish and in other hosts are conspecific. Further experimental cross-infections are currently being conducted by the author to determine if host species affects egg morphology.

Lake herring and chub (*Coregonus* spp.) populations have undergone disastrous declines in abundance and have even become extinct in the lower Great Lakes (Berst and Spangler 1972; Christie 1972; Hartman 1972; Wells and McLain 1972). Both hosts are indigenous and probably were important in propagating *C. farionis* populations in the Lower Great Lakes in the past. The reduced abundance of these hosts may explain the decline of *C. farionis* populations in the lower Great Lakes. *Cystidicola stigmatura* became rare or disappeared from the Great Lakes when lake trout populations collapsed (Black 1983a). Although lake herring numbers have been reduced in some regions of Lake Superior (Selgeby 1982), it still supports the only large herring fishery on the Great Lakes (Baldwin et. al. 1979). The success of introduced Pacific salmons and rainbow trout, which are suitable hosts for *C. farionis*, has ensured the survival of the worm in Lake Superior even if lake herring and chub numbers decline further. It is expected that in Lake Ontario, where Pacific salmons and rainbow trout have recently become abundant (Crossman and Van Meter 1979), *C. farionis* may again prosper. No swimbladder nematodes were found in 10 coho salmon from Lake Ontario examined in the present study nor in a variety of exotic salmonids examined by Moody (1981). Declines in lake herring and chub populations in Lake Ontario occurred prior to the successful establishment of exotic salmonids (Moody 1981). Moody (1981) found a small population of lake whitefish from the Bay of Quinte in the eastern end of Lake Ontario that was infected with small numbers of *C. farionis*. If these worms can mature in the introduced salmonids the possibility for widespread distribution of the worm within the lake still exists. The recent range extension of the pink salmon into the lower Great Lakes from Lake Superior (Emery 1981, Kwain and Lawrie 1981) might provide another mechanism for the dispersal of *C. farionis*, provided suitable intermediate hosts are encountered. Pink salmon are infected with large numbers of mature

worms in Lake Superior (Lankester and Smith 1980).

The infection of pygmy whitefish from Lake Superior represents a new host record for *C. farionis*. The pygmy whitefish is probably not a suitable host for *C. farionis* as none of the worms recovered was mature. Mudry and Anderson (1977) reported immature *Cystidicola* sp. (probably *C. farionis*) in pygmy whitefish from the Ottertail River, British Columbia, but specific identification was not made. The deepwater amphipod *Pontoporeia affinis* is an important item in the diet of pygmy whitefish from Lake Superior (Eschmeyer and Bailey 1955) and is likely the amphipod important in transmission of *C. farionis* to this fish.

Filaments occur on the eggs of a variety of cystidicolid nematodes (Ekbaum 1935; Choquette 1955; Ko and Anderson 1969; Margolis 1977; Appy 1981; Appy and Anderson 1982; Ko et. al. 1985). Smith (1978) suggested that the filaments on the eggs of *C. farionis* may increase the probability of eggs being ingested by the intermediate hosts through attachment of eggs to vegetation or to the feeding appendages of amphipods. Sandeman and Burt (1972) reported that the fine polar filaments on the egg of the cestode *Bothrimonus* sp. adhere quite strongly to vegetation. *Cystidicola farionis* eggs readily adhered to setose areas on the appendages of amphipods exposed in this study. Filaments on eggs became entangled in setae and adhered to flat surfaces on the gnathopods and antennae. These appendages are commonly used by amphipods to collect food (Marshall and Orr 1960; Barnes 1980) especially when foraging on surficial sediments. Adhesion of filaments to feeding appendages would certainly increase the chances of eggs being eaten by the intermediate host. Of the cystidicolids which have filamented eggs and whose life cycles are known, almost all utilize crustaceans, usually amphipods as intermediate hosts. Exceptions are *Cystidicoloides tenuissima* and *Spinitectus gracilis* which use burrowing mayfly nymphs as intermediate hosts (Choquette 1955;

Chubb 1982).

Instead of filaments, the eggs of *Cystidicola stigmatura* (=*Cystidicola cristivomeri*) have conspicuous lateral lobes (White and Cable 1942). These lobes may increase the chances of eggs being ingested by the intermediate host *Mysis relicta*. *Mysis relicta* is generally thought to prey on planktonic crustacea, especially cladocerans (Cooper and Goldman 1980; Lazenby and Fürst 1981). Lazenby and Langford (1970) found that *Mysis relicta* is opportunistic and its feeding habits vary in different lakes. In one Arctic lake they fed primarily on diatoms from 1 to 70 μm in diameter. In two Swedish lakes studied by Kinsten and Olsen (1981), *Mysis relicta* was a voracious predator at certain times of the year but fed on detritus, cysts and benthic diatoms when restricted to the bottom zone in early summer. Lobed eggs of *C. stigmatura* at 40 to 45 μm long (Ko and Anderson 1969) are similar in size and shape to many diatoms (see Patrick and Reimer 1966). Therefore, mysids may become infected by eating eggs directly or perhaps by consuming cladocerans which have ingested eggs of *C. stigmatura*. Kinsten and Olsen (1981) suggested that phytoplankton in the gut of mysids making vertical migrations may have come from the gut of zooplankton consumed by the mysids.

Although the success rate in establishing infections in amphipods using fresh eggs was initially high, the mortality of amphipods in the ensuing weeks was also high. Mortality of amphipods was in part due to temperature fluctuations but could not be attributed to *C. farionis* infections since infected and control amphipods experienced similar mortality. One fourth-stage larva from a single lake whitefish was the only swimbladder nematode recovered from 30 fish fed amphipods previously exposed to eggs of *C. farionis*. This nonetheless demonstrates for the first time that worms can be transmitted directly from amphipods to the definitive host.

Black and Lankester (1980) successfully infected rainbow trout with third-

stage larvae of *C. farionis* by administering worms to anaesthetized fish with a stomach tube. Fish frequently regurgitated after larvae were given in this way. Attempts to stomach tube *C. farionis* into rainbow trout in this study were unsuccessful and the technique was difficult to use on lake whitefish due to the small size of their mouth. By feeding rainbow trout and lake whitefish sections of earthworm into which *C. farionis* larvae were injected, fish were successfully infected with a minimal amount of stress and handling. The number of larvae administered to the fish can be easily controlled using this method and if regurgitation occurs, the larvae can be readministered to the fish. The success rate in infecting fish using the earthworm method was similar to that of Black and Lankester (1980) in infecting rainbow trout with *C. farionis* using a stomach tube.

The occurrence of two third-stage larvae of *C. farionis* in the esophagus of one lake whitefish examined 31 hours after infection supports the finding of Black and Lankester (1980) that *C. farionis* migrates to the swimbladder via the pneumatic duct. Migration times are probably similar in lake whitefish and rainbow trout. The development of *C. farionis* appeared more rapid in lake whitefish than in rainbow trout (Black and Lankester 1980; this study). Worms were quicker to moult and grew faster in lake whitefish. However, lake whitefish were held at higher temperatures than rainbow trout for the first 80 days after infection. Temperature has a pronounced effect on the development of *C. farionis* in amphipod intermediate hosts (Smith and Lankester 1979) and likely influences the rate of development in the definitive host. The rate of development of *C. farionis* in rainbow trout infected by Black and Lankester (1980) was similar to that in rainbow trout infected in this study and held at similar temperatures.

Examination of successive fall, spring and fall samples provides evidence that *C. farionis* infections change seasonally in lake whitefish. Fish from Pettit Lake

sampled in spring had higher intensities and a higher percentage of mature worms than fish taken in fall. The large proportion of immature worms in fall suggests that these worms have only recently been acquired. Most transmission, then, probably occurs in late fall and worms mature over winter given a conservative estimate of 5 to 6 months for worms to reach sexual maturity (see Black and Lankester 1980). Maximum egg production by *C. farionis* should occur in spring and early summer and most transmission to amphipods would probably occur at this time because of the short term viability of free eggs. Amphipods infected in the spring and early summer are not immediately important in transmission since there is little recruitment of worms to lake whitefish during the summer months. Adult worms must die over summer suggesting a life span up to one year.

Chubb (1982) states that seasonal feeding patterns of the final host are usually the most important factor in determining the seasonal cycles of nematodes in fresh water fishes. The distribution and feeding habits of amphipods will likely result in certain species of amphipods becoming infected with *C. farionis* at higher rates than others (Smith 1978). The distribution of the definitive hosts during periods of egg output will also determine which amphipod species become infected. Lake whitefish congregate in shallow waters to spawn in fall (Scott and Crossman 1973). In Pettit Lake, the shallow water *H. azteca* was the only amphipod present in stomachs of lake whitefish sampled in fall. *Hyalella azteca* is probably the most important amphipod species for transmission of worms in Pettit Lake as *C. farionis* is principally transmitted during fall. Infection of *H. azteca* may occur during periods of high egg output the previous spring or in early fall when lake whitefish return to shallow water. *Pontoporeia affinis* may be involved in transmission if lake whitefish feed in deep water after spawning.

The seasonal cycle of *C. farionis* infections in rainbow trout from Lake

Superior is similar to that in lake whitefish from Pettit Lake. Prevalence, intensity and the proportion of mature worms were higher in spring than in fall. Lankester and Smith (1980) found the proportion of mature worms in lake herring and lake whitefish from Lake Superior was highest in fall. Prevalence and intensity did not vary with season. These differences are likely due to differences in the feeding habits of the definitive hosts involved.

Several authors have reported on the seasonality of *C. farionis* infections. Lake whitefish in Cold Lake, Alberta harboured the largest numbers of *C. farionis* (= *C. stigmatura*) during the winter months (Leong and Holmes 1981). Larval parasites were found during all seasons suggesting transmission occurred throughout the year. Most mature worms were found in spring and early summer, with the bulk of transmission occurring in fall from amphipods infected over summer. Watson and Dick (1979) also found that *C. farionis* was most abundant in lake whitefish from Southern Indian Lake, Man. during the winter months. Awachie (1973) found no seasonal pattern in *C. farionis* infections in brown trout from Afon Terrig, Wales, but the prevalence of infection in the intermediate host *Gammarus pulex* was highest in fall and winter. Brown trout from the River Itchen, England had the highest prevalence and intensity of *C. farionis* during the winter months (Poynton 1985). Poynton (1985) attributed this to a lowered resistance to infection in winter and to the finding of Awachie (1973) that prevalence in the intermediate host was highest during fall and winter. It should be noted that the timing of life cycles in different locations can be quite different (Chubb 1982). Neither Awachie (1973) nor Poynton (1985) mention the proportion of mature worms or feeding habits of the definitive host. Both factors can have profound effects on seasonality.

Other cystidicolids are known to exhibit different seasonal patterns of infection. *Cystidicola stigmatura* (= *Cystidicola cristivomeri*) exhibits no seasonal

fluctuations as worms live to be at least ten years old (Black and Lankester 1981). Moravec (1971) found that *Cystidicoloides tenuissima* may have two generations per annum. The lack of seasonality of *Spinitectus gracilis* in Lake Opeongo yellow perch (*Perca flavescens* (Mitchill)) (Cannon 1973) was attributed to overlap of worm recruitment and mortality. A parasite may exhibit different seasonal cycles in different locations, or seasonality in one location, but not in another (Chubb 1982).

Age, length and sex of lake whitefish from Pettit Lake had no significant effect on intensity or the proportion of *C. farionis* reaching sexual maturity. Increasing age of lake whitefish from Cold Lake, Alta. (Leong 1975) and Southern Indian Lake, Man. (Watson and Dick 1979) was associated with increased abundance of *C. farionis*. This was attributed to increased predation on amphipods with age. Prevalence doubled in fish between their second and third year in Cold Lake, but after age 3, increasing age had no effect on the occurrence of *C. farionis* although intensities increased slightly (Leong 1975). Similarly, prevalence and intensity of *C. farionis* in brown trout examined by Poynton (1985) generally were highest in the oldest fish examined (2+). Effects of host age on intensity of *C. farionis* infections in lake whitefish from Pettit Lake were not apparent because of the small number of young fish (<5+) examined. None of these fish was infected. Lankester and Smith (1980) found that host length had a significant, positive effect on the intensity of *C. farionis* in lake whitefish from South Bay, Lake Huron, lake herring from Lake Nipigon, and pink salmon and rainbow smelt from Lake Superior.

The absence of *C. stigmatura* in lake trout from Pettit Lake is puzzling as it occurs in lake trout from Mount Lake to which Pettit Lake is connected. Although the number of lake trout sampled was small, it is unlikely that any Pettit Lake *C. stigmatura* infections were overlooked as this worm is frequently 100 percent prevalent within infected populations of lake trout (Black 1983c). The intermediate

host *Mysis relicta* is probably absent from Pettit Lake. This would prevent the establishment of *C. stigmatura* in the lake even if infected lake trout from Mount Lake immigrate into Pettit Lake. The lack of mysids in lake whitefish stomachs supports this view.

The absence of *C. farionis* in the swimbladder of a rainbow trout that was fed 20 rainbow smelt does not dismiss the possibility that rainbow smelt may act as a paratenic host in transmitting *C. farionis* to piscivorous salmonids. Twenty rainbow smelt that were examined contained only 18 infective larvae. The probability of this number of larvae producing an experimental infection is low when one considers that less than 5 percent of larvae administered with the earthworm technique were recovered. The success rate of larvae escaping from an ingested smelt and migrating to the swimbladder would be expected to be less than that for larvae escaping from an earthworm. Hnath (1969) demonstrated experimentally that brook trout (*Salvelinus fontinalis* Mitchill) could become infected with the acanthocephalan *Echinorhyncus salmonis* when sections of infected coho salmon intestine were eaten. The probability of their escape from the sections of intestine would be much greater than that from an entire fish. Larger numbers of rainbow smelt fed to several rainbow trout would demonstrate more accurately the ability of *C. farionis* to use rainbow smelt as paratenic hosts.

The swimbladder nematodes *C. farionis* and *C. stigmatura* exhibit some striking biological differences. The seasonal fluctuations in the numbers of *C. farionis* in many fish hosts suggests that it is relatively short-lived, perhaps a year or less. *Cystidicola stigmatura* may live for more than ten years (Black and Lankester 1981). The absolute fecundity (number of eggs in a female worm) of *C. farionis* is 10 to 20 times greater than that of *C. stigmatura.* The number of eggs increases with worm length in both species. Black (1984) found that the reproductive output (number of

free eggs in swimbladder) was related to worm length as well. If the assumption that absolute fecundity is related to reproductive output is correct, some striking differences exist in the reproductive strategies of these two species. The relatively low fecundity of *C. stigmatura* may be related to its long life span. Populations of *C. stigmatura* are relatively stable (usually 100 percent of hosts are infected with similar numbers of worms), exhibit density dependent regulatory mechanisms and do not have seasonal changes in abundance or maturity (Black and Lankester 1981). Prevalence and intensity of *C. farionis* populations tend to fluctuate seasonally and there is a lack of density dependent regulatory mechanisms (Lankester and Smith 1980). The differences in the biology of these two species become even more striking when one considers that speciation within the genus probably occurred after the onset of Wisconsin glaciation (Black 1983c).

The cephalic structures of cystidicolid nematodes are important in classification and their small size makes them very difficult to view and interpret (Chabaud 1975). As a result many of the genera have been poorly described (Appy and Anderson 1982). Terminology applied to the various cephalic structures has been inconsistent, augmenting the confusion surrounding the group. Although it is not always practical to use a scanning electron microscope to identify nematodes, the technique helps to elucidate morphological features seen using the light microscope (Appy and Anderson 1982). The recent work of several authors (Margolis 1977; Appy and Dadswell 1978; De and Moravec 1979; Appy 1981; Appy and Anderson 1982; Ko et. al. 1985) has demonstrated the value of the electron microscope in studying this group. Features of the buccal region of *Cystidicola* spp. can clearly be seen from scanning electron micrographs. Ko and Anderson (1969) were apparently mistaken in describing two lateral teeth and numerous small external teeth in the form of fine serrations for *Cystidicola* spp.

The conical apex of the anterior portion of the pseudolabium of *Cystidicola* is similar to that of most species of *Ascarophis* (Appy 1981). The anterolateral extension of the wall of the buccal capsule fused to the medial aspect of the pseudolabium in *Cystidicola* is probably homologous to the medial bidentate plates of *Capillospirura* (Appy and Anderson 1982) and the pseudolabial processes of *Pseudascarophis* (Ko et. al. 1985). The sublabial teeth of *Cystidicola* are similar in location and are probably homologous to the sublabia of *Cystidicoloides* (De and Moravec 1979), *Caballeronema* (Margolis 1977), *Ascarophis* (Appy 1981), and *Capillospirura* (Appy and Anderson 1982). Sublabia are wanting in *Pseudascarophis* and *Metabronema* (Ko et. al. 1985) although the digitiform buccal processes of *Pseudascarophis* are similar to the sublabial teeth found in *Cystidicola.* Submedian labia are lacking in these two genera as well as in *Cystidicoloides* (De and Moravec 1979). Further examination of additional species and genera will likely resolve some of the taxonomic confusion surrounding the cystidicolids and contribute to a better understanding of homologies leading to standardized terminology. Presently there are 15 or 16 recognized genera in the family (Ko et. al. 1985).

References

Amin, O. M. 1977. Helminth parasites of some southwestern Lake Michigan fishes. Proc. Helminth. Soc. Wash. 44: 210-217.

Anderson, R. C. 1958. Méthode pour l'examen des nématodes en vue apicale. Ann. Parasitol. Hum. Comp. 33: 171-172.

Anthony, J. D. 1963. Parasites of eastern Wisconsin fishes. Trans. Wisc. Acad. Sci. 52: 83-93.

Appy, R. G. 1981. Species of *Ascarophis* van Beneden, 1870 (Nematoda: Cystidicolidae) in North Atlantic fishes. Can. J. Zool. 59: 2193-2205.

Appy, R. G., and R. C. Anderson. 1982. The genus *Capillospirura* Skrjabin, 1924 (Nematoda: Cystidicolidae) of sturgeons. Can. J. Zool. 60: 194-202.

Appy, R. G., and M. J. Dadswell. 1978. Parasites of *Acipenser brevirostrum* LeSueur and *Acipenser oxyrhyncus* Mitchill (Osteichthyes: Acipenseridae) in the Saint John River estuary, N. B., with a description of *Caballeronema pseudoargumentosus* sp. n. (Nematoda: Spirurida). Can. J. Zool. 56: 1382-1391.

Awachie, J. B. E. 1973. Ecological observations on *Metabronema truttae* Baylis, 1935, and *Cystidicola farionis* Fischer v. Waldheim, 1798 (Nematoda, Spiruroidea) in their intermediate and definitive hosts in Affon Terrig. Acta

Parasitol. Pol. 21: 661-670.

Bailey, R. M., C. E. Bond, J. R. Brooker, E. A. Lachner, R. N. Lea, and W. B. Scott. 1980. A list of common and scientific names of fishes from the United States and Canada. Am. Fish. Soc. Spec. Publ. 12: 174 p.

Baldwin, N. S., R. W. Saalfield, M. A. Ross, and W. J. Buettner. 1979. Commercial fish production in the Great Lakes 1867-1977. Great Lakes Fish. Comm. Tech. Rep. 23: 187 p.

Bangham, R. V. 1955. Studies on fish parasites of Lake Huron and Manitoulin Island. Am. Midl. Nat. 53: 184-194.

Barnes, R. D. 1980. Invertebrate Zoology. <4th ed.> Saunders College/Holt, Rinehart and Winston, Philadelphia, PA, 1089 p.

Berst, A. H., and G. R. Spangler. 1972. Lake Huron: effects of exploitation, introductions, and eutrophication on the salmonid community. J. Fish. Res. Board Can. 29: 877-887.

Black, G.A. 1980. Migration and development of *Cystidicola* spp. (Habronematoidea) in their definitive hosts and the population biology of *C. cristivomeri* White, 1941 in *Salvelinus* spp. M. Sc. Thesis. Lakehead University, Thunder Bay, Ontario.

Black, G. A. 1983a. Taxonomy of a swimbladder nematode, *Cystidicola stigmatura*

(Leidy), and evidence for its decline in the Great Lakes. Can. J. Fish. Aquat. Sci. 40: 643-647.

Black, G. A. 1983b. A strain of *Cystidicola farionis* (Nematoda) as an indicator of lake trout of Bering ancestry. Can. J. Fish. Aquat. Sci. 40: 2034-2040.

Black, G. A. 1983c. Origin, distribution and postglacial dispersal of a swimbladder nematode, *Cystidicola stigmatura.* Can. J. Fish. Aquat. Sci. 40: 1244-1253.

Black, G. A. 1984. Reproductive output and population biology of *Cystidicola stigmatura* (Leidy) (Nematoda) in Arctic char, *Salvelinus alpinus* (L.) (Salmonidae). Can. J. Zool. 63: 617-622.

Black, G. A., and M. W. Lankester. 1980. Migration and development of swimbladder nematodes, *Cystidicola* spp. (Habronematoidea), in their definitive hosts. Can. J. Zool. 58: 1997-2005.

Black, G. A., and M. W. Lankester. 1981. The transmission, life span, and population biology of *Cystidicola cristivomeri* White, 1941 (Nematoda: Habronematoidea) in char, *Salvelinus* spp. Can. J. Zool. 59: 498-509.

Bousfield, E. L. 1958. Freshwater amphipod crustaceans of glaciated North America. Can. Field Nat. 72: 55-113.

Cannon, L. R. G. 1973. Diet and intestinal helminths in a population of perch, *Perca flavescens.* J. Fish Biol. 5: 447-457.

Casselman, J. M., J. J. Collins, E. J. Crossman, P. E. Ihssen, and G. R. Spangler. 1981. Lake whitefish (*Coregonus clupeaformis*) stocks of the Ontario waters of Lake Huron. Can. J. Fish. Aquat. Sci. 38: 1772-1789.

Chabaud, A. G. 1975. Keys to the genera of the order Spirurida. Part 2. Spiruroidea, Habronematoidea and Acuaroidea. *In* CIH Keys to the nematode parasites of vertebrates. No. 3. *edited by* R. C. Anderson, A. G. Chabaud, and S. Willmott. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England. pp. 29-58.

Choquette, L. P. E. 1955. The life history of the nematode *Metabronema salvelini* (Fujita, 1920) parasitic in the speckled trout, *Salvelinus fontinalis* (Mitchill), in Quebec. Can. J. Zool. 33: 1-4.

Christie, W. J. 1972. Lake Ontario: effects of exploitation, introductions and eutrophication on the salmonid community. J. Fish. Res. Board Can. 29: 913-929.

Chubb, J. C. 1982. Seasonal occurrence of helminths in freshwater fishes. Part IV. Adult Cestoda, Nematoda and Acanthocephala. Adv. Parasitol. 20: 1-193.

Conneely, J. J., and T. K. McCarthy. 1984. The metazoan parasites of freshwater fishes in the Corrib catchment area, Ireland. J. Fish Biol. 24: 363-375.

Cooper, S. D. and C. R. Goldman. 1980. Opossum shrimp (*Mysis relicta*) predation

on zooplankton. Can. J. Fish. Aquat. Sci. 37: 909-919.

Crossman, E. J., and H. D. Van Meter. 1979. Annotated list of the fishes of the Lake Ontario watershed. Great Lakes Fish. Comm. Tech. Rep. 36: 25 p.

De, N. C. and F. Moravec. 1979. Some new data on morphology and development of the nematode *Cystidicoloides tenuissima* (Zeder, 1800). Folia Parasitol. (Prague), 26: 231-238.

Dechtiar, A. O. 1972. Parasites of fish from Lake of the Woods, Ontario. J. Fish. Res. Board Can. 29: 275-283.

Ekbaum, E. 1936. Notes on the genus *Cystidicola* in Canadian fishes. Can. Field Nat. 50: 8-11.

Emery, L. 1981. Range extension of pink salmon (*Oncorhynchus gorbuscha*) into the lower Great Lakes. Fisheries 6(2): 7-10.

Eschmeyer, P. H., and R. M. Bailey. 1955. The pygmy whitefish, *Coregonus coulteri*, in Lake Superior. Trans. Am. Fish. Soc. 84: 161-199.

Fagerholm, H. P. 1982. Parasites of fish in Finland. VI. Nematodes. Acta Academiae Aboensis, B 40: 128 p.

Hartman, W. L. 1972. Lake Erie: effects of exploitation, environmental changes, and new species on the fishery resources. J. Fish. Res. Board Can. 29: 899-

912.

Hnath, J. G. 1969. Transfer of an adult acanthocephalan from one fish to another. Trans. Am. Fish. Soc. 98: 332.

Hunter, G. W., III, and R. V. Bangham. 1933. Studies on the fish parasites of Lake Erie. II. New Cestoda and Nematoda. J. Parasitol. 19: 304-311.

Ihssen, P. E., and J. S. Tait. 1974. Genetic differences in retention of swimbladder gas between two populations of lake trout (*Salvelinus namaycush*). J. Fish. Res. Board Can. 31: 1351-1354.

Ihssen, P. E., D. O. Evans, W. J. Christie, J. A. Reckahn, and R. L. DesJardine. 1981. Life history, morphology, and electrophoretic characteristics of five allopatric stocks of lake whitefish (*Coregonus clupeaformis*) in the Great Lakes region. Can. J. Fish. Aquat. Sci. 38: 1790-1807.

Jones, F. R. H., and N. B. Marshall. 1953. The structure and functions of the teleostean swimbladder. Biol. Rev. 28: 16-83.

Kennedy, C. R. 1974. A checklist of British and Irish freshwater fish parasites with some notes on their distribution. J. Fish. Biol. 6: 613-644.

Kinsten, B., and P. Olsen. 1981. Impact of *Mysis relicta* Löven introduction on the plankton of two mountain lakes, Sweden. Rep. Inst. Freshw. Res., Drottningholm 59: 64-74.

Kirkpatrick, M., and R. K. Selander. 1979. Genetics of speciation in lake whitefishes in the Allegash basin. Evolution 33: 478-485.

Ko, R. C., and R. C. Anderson. 1969. A revision of the genus *Cystidicola* Fischer, 1798 (Nematoda: Spiruroidea) of the swimbladder of fishes. J. Fish. Res. Board Can. 26: 849-864.

Ko, R. C., L. Margolis, and M. Machida. 1985. *Pseudascarophis kyphosi* n. gen., n. sp. (Nematoda: Cystidicolidae) from the stomach of the fish *Kyphosus cinerascens* (Forskål) from Japan. Can. J. Zool. 63: 2684-2688.

Kwain, W., and A. H. Lawrie. 1981. Pink salmon in the Great Lakes. Fisheries 6(2): 2-6.

Lankester, M. W., and J. D. Smith. 1980. Host specificity and distribution of the swimbladder nematodes *Cystidicola farionis* Fischer, 1798 and *C. cristivomeri* White, 1941 (Habronematoidea), in salmonid fishes of Ontario. Can. J. Zool. 58: 1298-1305.

Lazenby, D., and M. Fürst. 1981. Feeding of *Mysis relicta* Löven on macroplankton. Rep. Inst. Freshw. Res., Drottningholm 59: 64-74.

Lazenby, D., and R. R. Langford. 1973. Feeding and assimilation of *Mysis relicta.* Limnol. Oceanogr. 18: 280-285.

Leong, T. S. 1975. Metazoan parasites of fishes of Cold Lake, Alberta: a community analysis. Ph. D. Thesis, University of Alberta, Edmonton, Alberta.

Leong, T. S., and J. C. Holmes. 1981. Communities of metazoan parasites in open water fishes of Cold Lake, Alberta. J. Fish. Biol. 18: 693-713.

Margolis, L. 1977. *Caballeronema* gen. nov. for *Metabronema wardlei* Smedley, 1934 (Nematoda, Spiruroidea) from the marine fish *Scorpaenichthys marmoratus* from the Pacific coast of Canada. Excerta Parasitologica en Memoria del Doctor Eduardo Caballero y Caballero. Univ. Nac. Auton. Mex., Inst. Biol. Publ. Espec. 4: 447-454.

Margolis, L., and J. R. Arthur. 1979. Synopsis of the parasites of fishes of Canada. Bull. Fish. Res. Board Can. 199: 269 p.

Margolis, L., G. W. Esch, J. C. Holmes, A. M. Kuris, and G. A. Schad. 1982. The use of ecological terms in parasitology. J. Parasitol. 68: 131-133.

Marshall, S. M., and A. P. Orr. 1960. Feeding and nutrition. p. 227-258, *in* T. H. Waterman <ed.> The physiology of the crustacea, vol. I. Academic Press Inc., New York, 670 p.

Moody, T.M. 1981. Experimental studies of the nematode *Cystidicola farionis* Fischer, 1798, and an examination of Lake Ontario fishes. H. B. Sc. Thesis, Lakehead University, Thunder Bay, Ontario.

Moravec, F. 1971. Studies on the development of the nematode, *Cystidicoloides tenuissima* (Zeder, 1800). Vestn. Cesk. Spol. Zool. 35: 43-55.

Mudry, D. R., and R. S. Anderson. 1977. Helminth and arthropod parasites of fishes in the mountain national parks of Canada. J. Fish Biol. 11: 21-33.

Patrick, R., and C. W. Reimer. 1966. The diatoms of the United States exclusive of Alaska and Hawaii, vol. I. Monogr. Acad. Nat. Sci. Phila. 13: 688 p.

Poynton, S. L. 1985. Parasites of trout: Epizootiology and transmission in the River Itchen and three of its fish farms. Ph. D. Thesis, University of Southampton, Southampton, England.

Sandeman, I. M., and M. D. B. Burt. 1972. Biology of *Bothrimonus* (= *Diplocotyle*) (Pseudophyllidea: Cestoda): ecology, life cycle, and evolution; a review and synthesis. J. Fish. Res. Board Can. 29: 1381-1395.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 966 p.

Selgeby, J. H. 1982. Decline in lake herring (*Coregonus artedii*) in Lake Superior: an analysis of the Wisconsin herring fishery, 1936-78. Can. J. Fish. Aquat. Sci. 39: 554-563.

Skinker, M. S. 1931. A redescription of *Cystidicola stigmatura* (Leidy), a nematode parasitic in the swimbladder of salmonoid fishes and a description of a new

nematode genus. Trans. Am. Microscop. Soc. 50: 372-379.

Smith, J. D. 1978. Studies of the biology of swimbladder nematodes, *Cystidicola* spp. (Habronematoidea). M. Sc. Thesis, Lakehead University, Thunder Bay, Ontario.

Smith, J.D., and M. W. Lankester. 1979. Development of swimbladder nematodes (*Cystidicola* spp.) in their intermediate hosts. Can. J. Zool. 57: 1736-1744.

Steele, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics. McGraw-Hill Book Company, New York.

Valtonon, E. T., and T. Valtonen. 1978. *Cystidicola farionis* as a swimbladder parasite of the whitefish in the Bothnian Bay. J. Fish Biol. 13: 557-561.

Warren, B. 1952. Report of parasites from the Lake Superior cisco, *Leucichthys artedi arcturus.* J. Parasitol. 38: 495.

Watson, R. A., and T. A. Dick. 1979. Metazoan parasites of whitefish, *Coregonus clupeaformis* (Mitchill) and cisco, *C. artedii* LeSueur from Southern Indian Lake, Manitoba. J. Fish Biol. 15: 579-587.

Wells, L., and A. L. McLain. 1972. Lake Michigan: effects of exploitation, introductions, and eutrophication on the salmonid community. J. Fish. Res. Board Can. 29: 889-898.

White, F. M., and R. M. Cable. 1942. Studies on the morphology of *Cystidicola cristivomeri* sp. nov. (Nematoda: Thelaziidae) from the swimbladder of *Cristivomer namaycush* (Walbaum). Am. Midl. Nat. 28: 416-423.