AN ABSTRACT OF THE THESIS OF

Gary Alan Gebhardt (Name) for the M. S. (Degree) in Fisheries (Major)

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Title STUDIES ON THE MOLLUSCAN AND FISH HOSTS OF THE "SALMON POISONING" FLUKE, NANOPHYETUS SALMINCOLA (Chapin)

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This study[1] was undertaken: (1) to obtain information on the distribution of the snail, Oxytrema silicula, in three coastal rivers in Oregon, and the seasonal incidence of infection in these snails and in snails from an inland stream, with the cercariae of the trematode, Nanophyetus salmincola; (2) to follow cercarial development in the snail under natural and experimental conditions; (3) to determine the species of animals naturally infected with the metacercariae and those susceptible to experimental infection; and (4) to follow development of the metacercariae in the fish hosts.

The snail was found widely distributed in the Alsea, Siletz, and Yaquina Rivers both in fresh and brackish water. Salinities in the brackish water areas were as high as 11.2 parts per thousand.

[1] This study was supported in part by Agricultural Experiment Station Project No. 393, and Public Health Service Research Grant 1 RO1 AI06599-01, from the National Institute of Allergy and Infectious Diseases.

Large snails were observed in all rivers during each season of the year. The incidence of infection with N. salmincola was highest in snails with aperture diameters from 10 to 13 mm, and varied from 27 percent in snails from the Alsea River to 29 percent in snails from the Siletz, and Yaquina Rivers. Thirty-five percent of snails from Oak Creek, a stream near Corvallis, were found infected with cercariae of N. salmincola. There was no apparent change in the intensity of infection in the snails during the study period. Immature, but not mature, cercariae of N. salmincola were found in snails during the months of December through March. Identity of these cercariae as N. salmincola was shown by holding infected snails at room temperature for 15 days. At the end of this time 90 percent of the snails infected with N. salmincola contained mature cercariae. Mature cercariae were noted in snails during the months of April through November.

The kidneys from 116 of 152 ocean-caught coho salmon, Oncorhynchus kisutch, and from 11 of 15 ocean-caught chinook salmon, O. tshawytscha, were found infected with 1 to 2400 metacercariae of N. salmincola. A dog fed two of these kidneys developed "salmon poisoning" disease. These results demonstrated for the first time that salinity was without any "cleansing" effect on either the trematode or the etiologic agent, though this effect was previously believed to occur.

The fishes Cottus perplexus, Lampetra richardsoni, L. tridentata, and Richardsonius balteatus, and the Pacific giant salamander, Dicamptodon ensatus, all from western Oregon streams, were found naturally infected with the metacercariae of N. salmincola. This is the first report of natural infections in an animal other than a fish and in nonsalmonid fishes. Fourteen species of fishes were experimentally infected: Salmo gairdneri, S. salar, S. trutta, Salvelinus fontinalis, S. namaycush, L. richardsoni, C. perplexus, Carassius auratus, R. balteatus, Catostomus macrocheilus, Lepomis macrochirus, Gasterosteus a. aculeatus, G. a. microcephalus, and Gambusia affinis. This extends the number of salmonid and nonsalmonid fishes susceptible to experimental infection. Cysts from all five of the naturally infected animals and from 12 of the 14 experimentally infected fishes were given to hamsters by stomach tube. The identification of the parasites as N. salmincola was confirmed by recovery of adult flukes from the hamsters in all instances, except one (probably because of low dosage).

Metacercariae from 1 to 106 days old from the experimentally infected fishes, and from naturally infected animals were studied. The stylet was absent in metacerariae approximately 50 days and older. The excretory bladder was always filled with round granules in metacercariae 15 days and older. The diameter of the cyst and thickness of the cyst wall increased with age of the parasite.

STUDIES ON THE MOLLUSCAN AND FISH HOSTS OF THE "SALMON POISONING" FLUKE, NANOPHYETUS SALMINCOLA (CHAPIN)

by

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INTRODUCTION

"Salmon poisoning", a disease of canids occurs only in western Oregon, northwest California, and southwestern Washington. Suckley (1855) was the first to report it. Pernot (1911) first demonstrated the infectious nature of "salmon poisoning". He transmitted the disease to dogs either by feeding infected trout kidney or by subcutaneous injection of blood from a sick dog. He also recognized the presence of parasites in fish kidneys, but incorrectly described them as freshwater amebae. Donham (1925a and b) found an intestinal trematode in dogs suffering from "salmon poisoning" disease and found an encysted form (the metacercariae) of the parasite in the muscels of "soreback" salmon. He was the first to establish a relationship between the presence of the fluke and occurrence of the disease in dogs.

Donham, Simms and Miller (1926) showed that the disease was transmitted by the fluke but were unable to characterize the etiologic agent. Simms and Muth (1933) suggested that the etiologic agent was either a rickettsia or a hemosporidian. Cordy and Gorham (1950) were the first to describe the agent and classified it as a rickettsia. The agent was named Neorickettsia helminthoeca by Philip, Hadlow

and Hughes (1953). The subject of "salmon poisoning" disease was reviewed by Philip (1955).

The fluke was named Nanophyes salmincola by Chapin (1926), who later (1928) changed the generic name to Nanophyetus. This species and N. schikhobalowi Skrjabin and Podjapolskaja (1931) from Siberian aborigines, was subsequently transferred to Troglotrema Odhner, by Witenberg (1932). Wallace (1933) considered Troglotrema for a related fluke, Sellacotyle mustelae Wallace, and retained the genus Nanophyetus for salmincola.

Nanophyetus salmincola requires three hosts for the completion of its life cycle: a freshwater mollusc, freshwater fishes, and fish-eating mammals such as canids, raccoons, mink, lynx, and the skunk (Pernot, 1911; Donham, 1925a and b; Donham, Simms and Miller, 1926; Donham and Simms, 1927; Simms et al., 1931, and Simms, Donham and Shaw, 1931). The molluscan host is Oxytrema (=Goniobasis) silicula Gould, a freshwater stream snail found only up to the Olympic Peninsula in Washington, west of the Cascade Mountains in Oregon, and in northern California (Simms, Donham and Shaw, 1931). Donham (1928) showed that the distribution of the molluscan host corresponded with the distribution of cases of "salmon poisoning" disease in dogs.

Seasonal variation in the incidence of infection in snails was first noted by Donham (1928). However, he observed several

different species of cercariae in the snails and did not correctly identify those of N. salmincola. Although there is some confusion as to the parasite stage he described, Sinitsin (1930) is credited with correct identification of the cercariae of N. salmincola. Simms et al. (1931) examined several hundred snails during each season of the year and showed that snails under 1.8 cm in length (based on the first three whorls) did not usually contain mature cercariae of N. salmincola. Simms, Donham and Shaw (1931) studied snails in the streams of Western Oregon each month of the year. They found that large specimens, 2.5 cm or more in total length, were scarce in fall and winter, but plentiful in late spring and early summer. The number of snails infected with many different species of cercariae decreased in late summer and fall coincident with a decrease in the number of large snails. They did not find any cercariae in snails less than 2.0 cm in total length. According to Bennington (1951), 51 of 93 snails he collected from the Alsea River in Western Oregon in February were over 3.0 cm in total length, but none were infected with N. salmincola. In March, 27 of 38 snails he collected from the same locality were 2 - 3 cm in length and none were infected with N. salmincola. Also in March, he collected a large number of snails from Oak Creek, a stream near Corvallis, of these, 1 was 3.5 cm in length, 22 were over 3 cm in length, 122 were 2-3 cm in length and 54 were less than 2 cm in length. None of these shed

N. salmincola cercariae and all but two were infected with the cercariae of some other trematode. He collected a second group of snails from Oak Creek 18 days later with measurements as follows: one was 3.6 cm, three were 3.5 cm., 22 were over 3 cm, 33 were 2-3 cm, 35 were 1-2 cm and 77 were less than 1 cm. Eleven of these, all over 3 cm in length, shed N. salmincola cercariae. Seventeen snails (length not given) did not shed cercariae, but were found by examination to be infected with N. salmincola. All of the others 2 cm or larger were infected with the cercariae of some other trematode. Bennington (1951) examined more than 100 snails and never found N. salmincola as part of a mixed infection. He did not determine the monthly incidence of N. salmincola infection in snails. Bennington and Pratt (1960) observed that the number of infected snails were high in contrast to the number which shed cercariae. They suggested that this difference could be attributed to the long period of time required for cercarial maturation.

Pernot (1911) was the first to observe the larval parasite in fish. He described the metacercariae as "minute whitish cysts" located along the backbone of salmon and trout. However, he mistakenly considered them to be amebae that "resembled round ova-like bodies with a more or less opaque center". Donham (1928) also observed the parasite in salmonid fish and correctly identified them as metacercariae in 1925. According to the early investigators

(Pernot, 1911; Donham, 1928; and Donham, Simms and Miller, 1926), salmon which became infected with N. salmincola in the streams before or during migration to the ocean were "cleansed" or lost the parasite during residence in the ocean. They stated that salmon became reinfected in the stream during their return migration to the spawning grounds. These conclusions were based on examination of a small number of ocean-caught salmon, and that susceptible dogs acquired the disease after eating salmon that had been in fresh water, but not after eating ocean-caught salmon. More recently, Bennington and Pratt (1960) found metacercariae of N. salmincola in a salmon reportedly taken at sea, and Farrell and Lloyd (1962) found metacercariae in three of five coho salmon and 19 of 27 chinook salmon caught near the mouth of the Columbia River. Farrell, Lloyd and Earp (1964) showed persistence of metacercariae and infectivity of the etiologic agent, N. helminthoeca, in coho salmon kept captive for 12, 24 and 33 1/2 months in ocean water. They also observed viable metacercariae in the kidneys of a five-year-old chinook salmon that had spent four years in the ocean.

On the basis of the initial work of Donham, Simms and Miller (1926), Simms et al. (1931), Simms, Donham and Shaw (1931), and in the absence of reports to the contrary, it has long been believed that salmonids were the only species of fish capable of serving as second intermediate hosts for N. salmincola. Donham, Simms and

Miller (1926) did not find this parasite in the following non-salmonid fishes (scientific names were not given) taken in western Oregon: two whitefish, two smelt, one chub, one sea bass, one silver perch, and five suckers. Simms et al. (1931) were unable to find the parasite in the following nonsalmonid fishes (numbers examined not given) from western Oregon streams: Micropterus dolomieui, smallmouth bass; Thaleichthys pacificus, eulachon or Columbia River smelt; Catostomus macrocheilus, largescale sucker; Mylocheilus caurinus, peamouth; and Acipenser transmontanus, white sturgeon. Pernot (1911) examined one mudcat (scientific name not given) and found it uninfected. The only attempts to experimentally infect nonsalmonid fishes with cercariae of N. salmincola were those of Bennington and Pratt (1960). They infected two speckled dace, Rhinichthys osculus, one riffle sculpin, Cottus gulosus (probably perplexus in view of present knowledge of the distribution of these fishes in Oregon), and two goldfish, Carassius auratus, by placing fish and infected snails together in aquaria.

Donham, Simms and Miller (1926) gave 140 μ as the approximate diameter of encysted metacercariae in fish, but noted that cysts from the same fish varied in size. Ward and Mueller (1926) observed nearly spherical cysts with diameters ranging from 166 to 246 μ in "black spotted trout" 40 to 50 mm long. Price (1929) stated that metacercariae occur in minute cysts about 140 μ in diameter.

Simms et al. (1930) stated that cysts enclosing living metacercariae were 170 to 255 μ in diameter with an average of 205 μ. They noted that metacercariae in the smaller cysts had unfilled excretory bladders, whereas, the bladders of the larger ones were filled. They concluded that the smaller cysts were younger or of more recent origin and subsequently substantiated this by examination of cysts from a recently experimentally infected rainbow trout. Bennington (1951) noted that the cyst wall of young cysts was thin and transparent, easily ruptured, and that the excretory bladder of young metacercariae was filled almost at once, after formation of the cyst with small round granules that made it appear white by reflected light and black by transmitted light. He observed that the excretory bladder increased in size and the stylet gradually disappeared as the metacercariae grew older. He found that presumably older encysted metacercariae from a wild fish, compared with younger ones, had a tougher and thicker cyst wall were larger in size, and the stylet was absent. The measurements given by Bennington (1951) for the excysted older metacercariae were: length 537 μ, width 275 μ, and the excretory bladder 250 x 180 μ.

This study was undertaken: (1) to determine distribution of O. silicula in three coastal rivers in Oregon, and the seasonal incidence of N. salmincola infection in these and in snails from an inland

stream; (2) to follow cercarial development in the snail under natural and experimental conditions; (3) to determine the species of animals naturally infected with the metacercariae and those susceptible to experimental infection; and (4) to follow metacercarial development in freshwater animals. The remaining parts of the thesis are subdivided into these four categories.

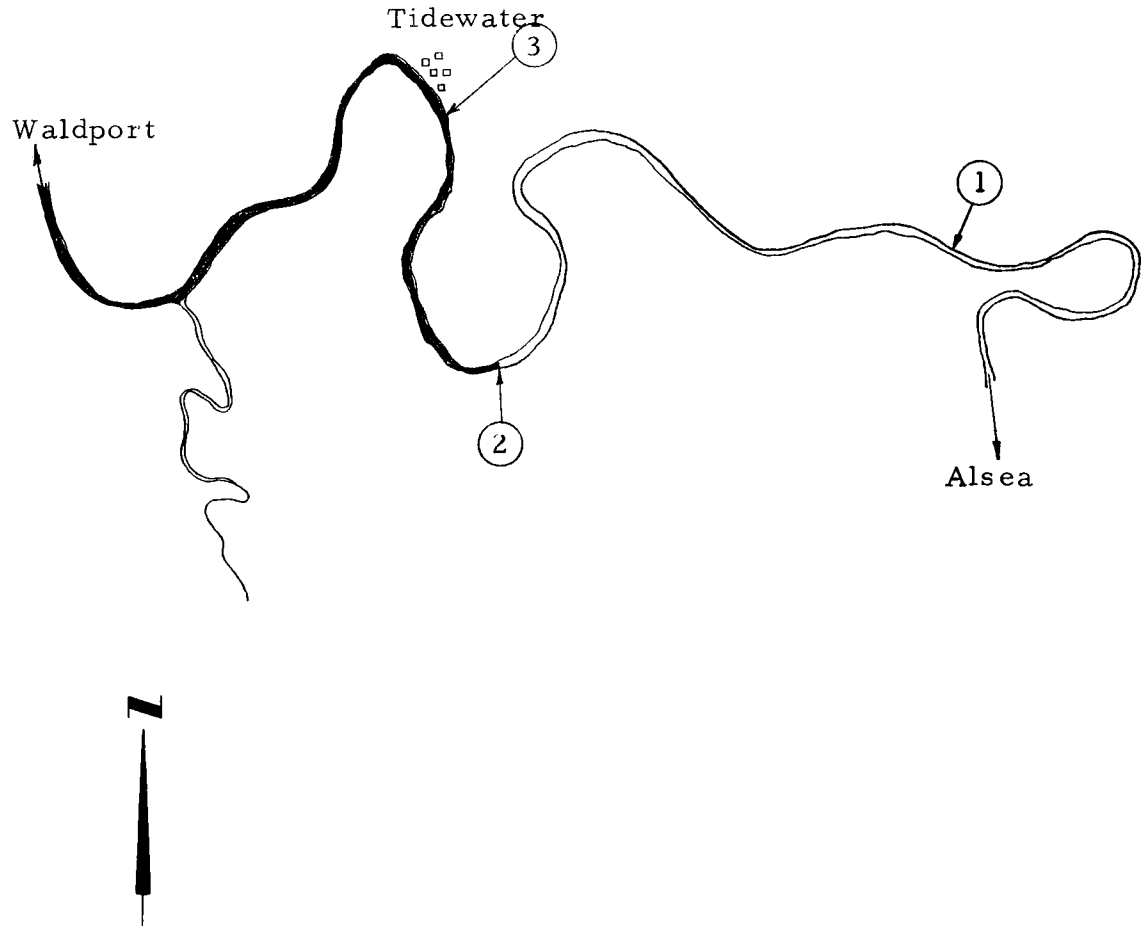
MATERIALS AND METHODS

I. Distribution of O. silicula in Three Coastal Rivers, and Seasonal Incidence of Infection with N. salmincola in These and in Snails from an Inland Stream

Snails were collected from the following Oregon coastal rivers: the Alsea, Siletz, and Yaquina (including its tributaries, Mill Creek and the Big Elk River), and from Oak Creek, a tributary to the Marys River, from August 1964 to June 1965 except during periods of high and cloudy water in late December 1964 and January 1965. Specimens were taken from fresh and brackish water in each of the three coastal rivers. Collection sites are shown in Figures 1-4. Temperatures and salinities (taken at or near high tide) at each collection site were recorded.

Incidence of infection with N. salmincola in snails collected from the Alsea, Siletz, and Yaquina Rivers was determined from August 1964 to May 1965. Snails from Oak Creek were examined for infection from January to June 1965.

The maximum aperture diameter, measured with vernier calipers, was used to indicate size of the snails rather than the total length or length of the first three whorls. The former method affords more uniform results than the latter methods since the spires of many snails are broken.



Scale: 1 inch = 1 mile

Figure 1. Map of part of the Alsea River drawn from the United States Geodetic Survey map of the Tidewater quadrangle. The circled numbers designate snail collection sites. The head of tidewater is located at the junction of shaded and unshaded parts of the river.

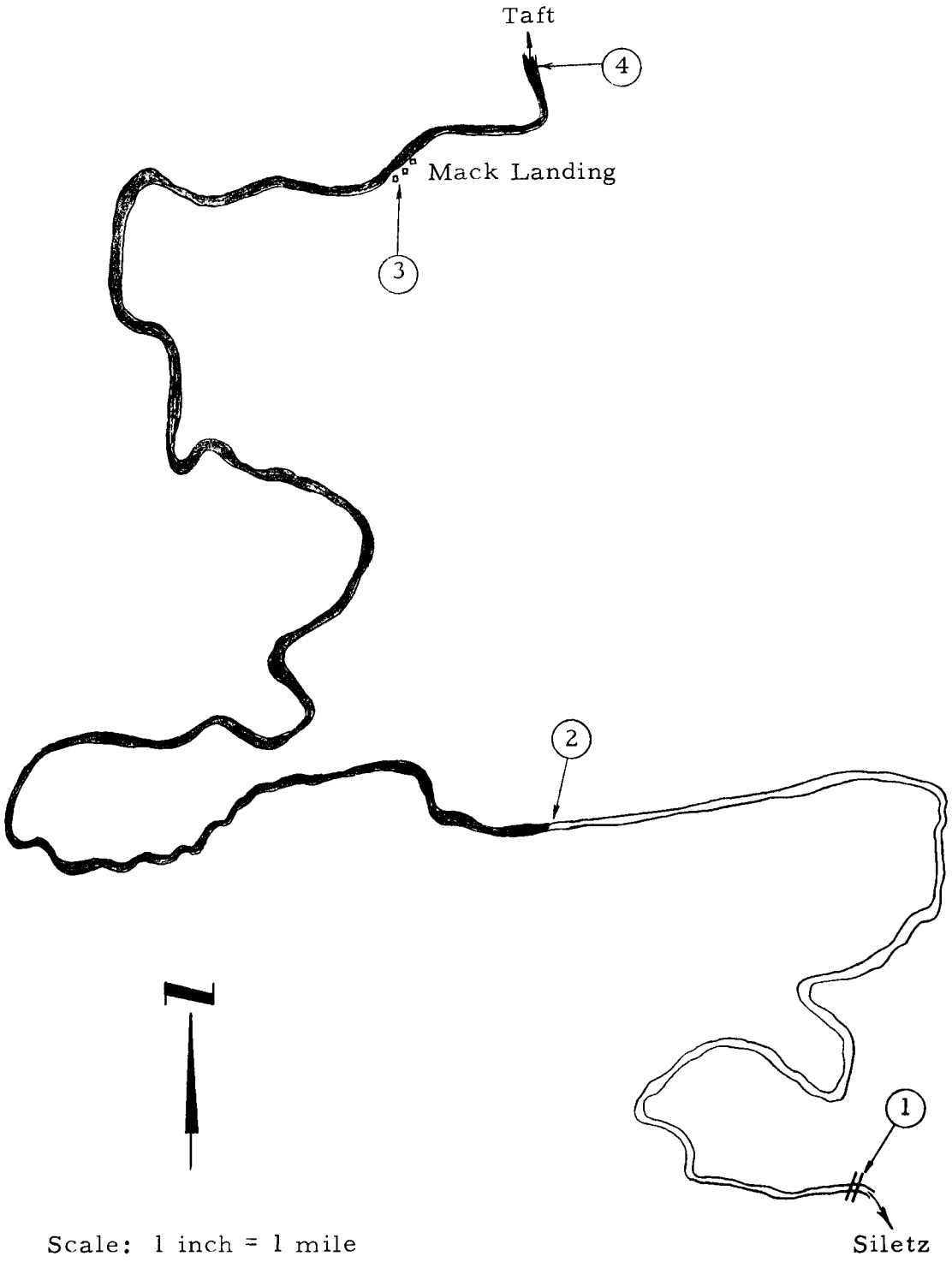
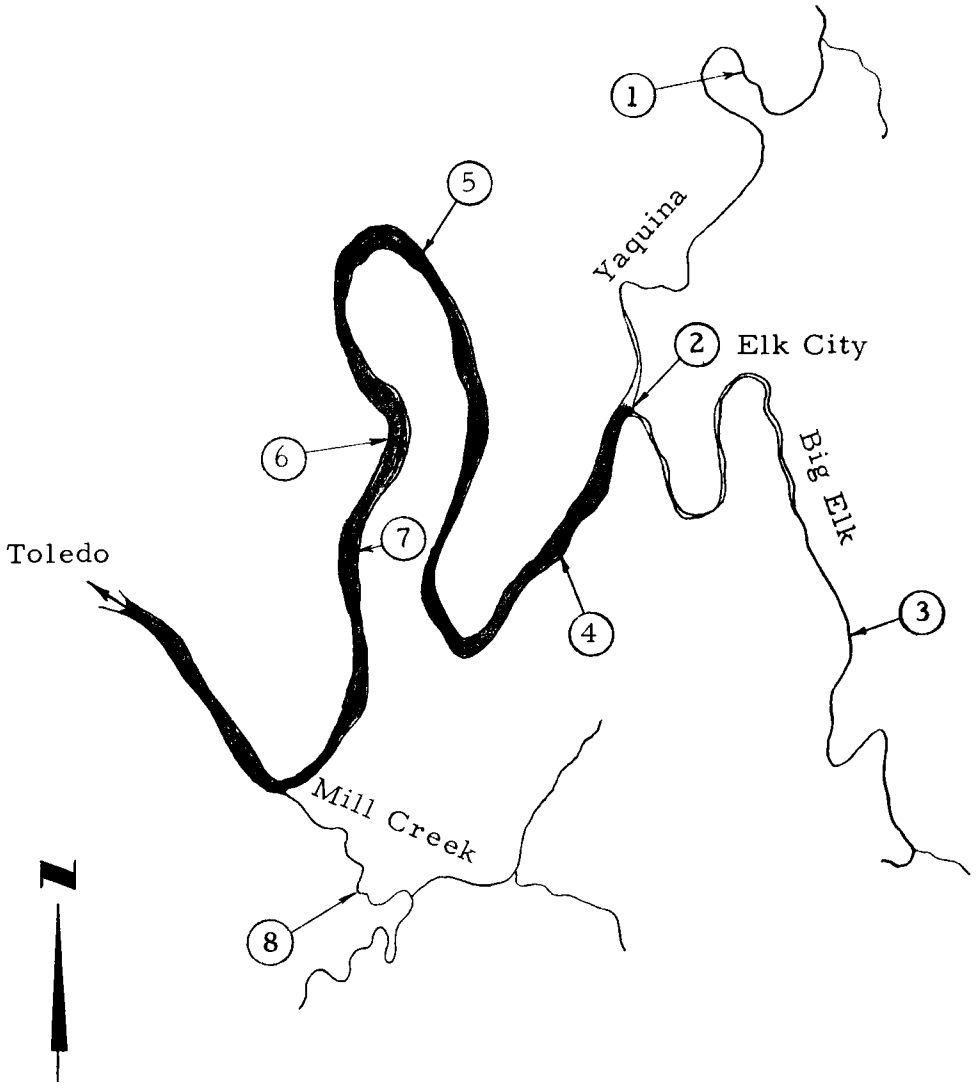
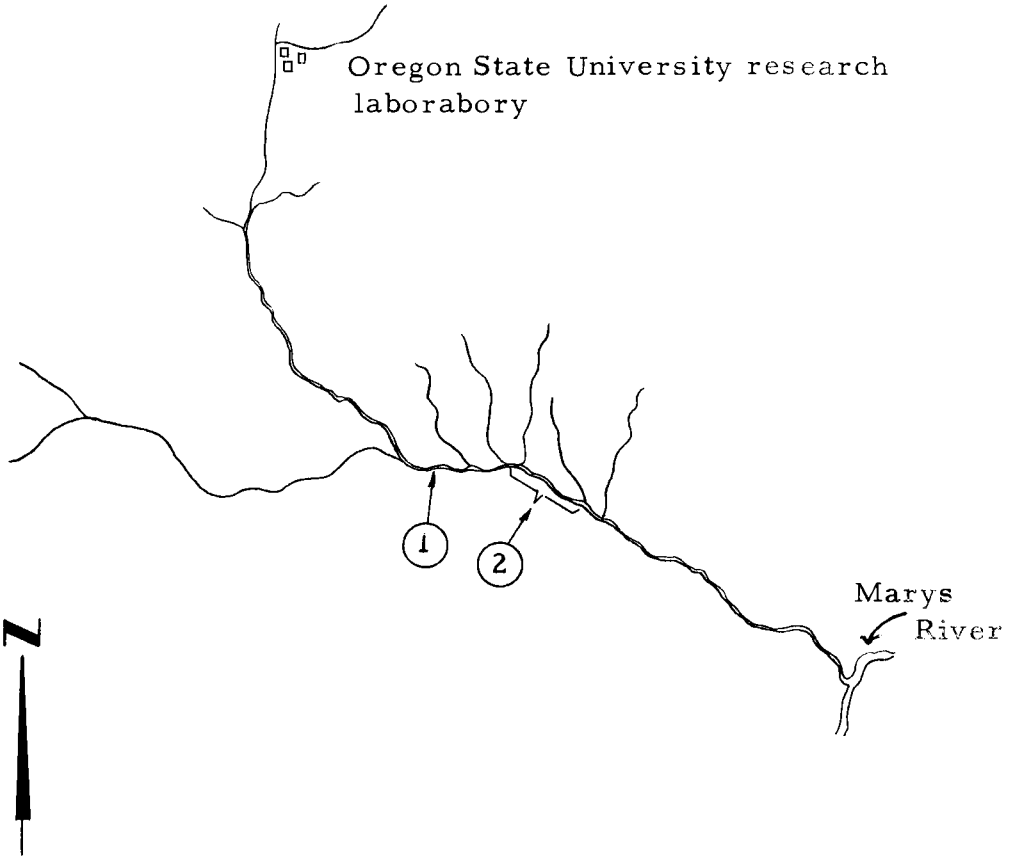


Figure 2. Map of part of the Siletz River drawn from United States Geodetic Survey map of the Siletz quadrangle. See legend for Figure 1 for further explanation.



Scale: 1 inch = 1 mile

Figure 3. Map of part of the Yaquina River system drawn from the United States Geodetic Survey map of the Toledo quadrangle. See Figure 1 for further explanation.



Scale: 1 inch = 1 mile

Figure 4. Map of part of Oak Creek, a tributary to the Marys River, drawn from United States Geodetic Survey map of the Corvallis quadrangle. Snail collection sites shown by circled numbers.

II. Development of Cercariae of *N. salmincola* in *O. silicula* under Natural and Experimental Conditions

Natural Conditions

Monthly collections of snails were made from the Alsea, Siletz, and Yaquina Rivers and cercarial development in these snails was followed from the first week of December 1964 to 1 April 1965. Weekly collections of snails were also made during this period from Oak Creek. Temperatures and salinities were recorded at each collection site. All snails were examined individually by crushing.

Experimental Conditions

Four experiments were conducted from 1 December 1964 to 1 April 1965 to determine the effect of temperature on development of the parasites in snails. In each of three experiments approximately 200 snails collected from the Alsea, Siletz, and Yaquina Rivers on 29 January 1965, were placed in aerated aquaria with standing water and held at room temperature (20-22 C) until 1 March 1965. The aperture diameters of these snails ranged from 6.0 to 11.5 mm. Ten to twenty-five snails were removed from each aquaria at one to three day intervals during the above period and examined individually by crushing. In the other experiment 390 snails, with aperture diameters from 6.0 to 13.0 mm, were collected from Oak Creek 3 March

1965, divided into three equal groups, and placed into five-gallon aquaria with standing water in a 4 C constant temperature room. The aquaria temperatures were maintained at 10, 16 and 22 C using aquaria heaters. Five to twenty-five snails from each of the above groups were examined individually by crushing at three to five day intervals from 3 March to 24 March 1965. Examinations of 25 snails from Oak Creek, and 15 each from the Alsea, Siletz, and Yaquina Rivers at the beginning of the experiments showed that only immature cercariae, presumably N. salmincola, were present. All snails were fed beet greens and lettuce weekly.

III. Natural and Experimental Infections of Marine and Freshwater Animals with the Metacercariae of N. salmincola

Natural Infections

Freshwater Animals

Dicamptodon ensatus, the Pacific giant salamander, and the following adult nonsalmonid fishes were obtained from Oak Creek, a stream near Corvallis: Lampetra richardsoni, brook lamprey; Cottus perlexus, reticulate sculpin; Richardsonius balteatus, redside shiner; and Catostomus macrocheilus, large-scale sucker. Adult Lampetra tridentata, Pacific lampreys, were collected from the Alsea River in western Oregon. Adult Gasterosteus a. aculeatus, brackish water threespine sticklebacks, were collected from Yaquina Bay, Oregon, and adults of the freshwater subspecies, G. a. microcephalus, from Eckman Lake, near Waldport, Oregon. Adult

affinis, mosquitofish, were obtained from farm ponds near Corvallis; adult Carassius auratus, goldfish, and juvenile Lepomis macrochirus, bluegills, from farm ponds near St. Paul, Oregon; and adult Perca flavescens, yellow perch, from Ten Mile Lakes, Lakeside, Oregon. Adult Thaleichthys pacificus, eulachon or Columbia River smelt, taken commercially near the mouth of the Lewis River, Washington, were purchased from a Portland fish dealer.

The animals were examined individually for infection, either by dissection or by a homogenization-sedimentation technique developed by Mr. Peter A. Nyberg (Department of Veterinary Medicine, Oregon State University). This latter method was rapid and efficient in detecting even light infections. The entire animal, or a selected part, was placed in a blender with 200 ml of water and blended for one minute. The homogenate was washed through a sieve having a standard screen scale of 65 meshes to the inch into a larger finger bowl and allowed to settle for one minute. The supernatent was decanted and the settled material diluted to 1000 ml with water and allowed to settle in a pharmaceutical flask for one minute. This last step was repeated and the resulting sediment examined for cysts.

The number of cysts per animal was determined either by a total count or from an average of five 0.1 ml aliquot samples of a 50 ml homogenate.

Cysts were administered to hamsters by stomach tube. The animals were killed approximately six days later, and the small

intestine examined for adult flukes. The hamster studies described here and under experimental infections were carried out by Mr. Peter Nyberg.

Marine Fishes

Kidneys from 152 Oncorhynchus kisutch, coho salmon, two O. gorbuscha, pink salmon, and 15 O. tshawytscha, chinook salmon, were examined for metacercariae of N. salmincola by the homogenization-sedimentation technic described above. The adult salmon were caught one to six miles off the central Oregon coast. The sex, weight, and length was recorded for each fish, and scales were taken to determine age of the fish. The number of cysts per kidney was determined either by a total count, or from an average of five 0.1 ml aliquot samples of a 50 ml homogenate.

Two infected coho salmon kidneys were fed to a nine-month-old female, kennel-raised dog. Daily body temperatures were recorded and examination of the feces for eggs of N. salmincola were made. The small intestine of the animal was examined at necropsy 17 days after exposure for adult flukes. This part of the study was carried out by Dr. Stuart Knapp, Department of Veterinary Medicine, Oregon State University.

Experimental Infections

All of the above species of fish (listed in section III) except the river lamprey, the eulachon, and the salamander were exposed experimentally, as were also the following salmonids, obtained from Oregon Game Commission hatcheries outside the enzootic area: 14- and 26-month-old Salmo salar, Atlantic salmon, from the Wizard Falls Hatchery; and 18-month-old Salvelinus namaycush, lake trout, three-month-old Salmo trutta, brown trout, and three-month-old Salvelinus fontinalis, brook trout, all from the Klamath Hatchery.

Twenty-six -month-old Salmo gairdneri, rainbow trout, from the Wizard Falls hatchery, and five-day-old laboratory-reared steelhead trout (anadromous rainbow trout), both of which are known to be susceptible to infection by the parasite, were used as positive controls to indicate infectiousness of the cercariae. Fish of each species were examined either by dissection or by homogenization before the start of the experiment to detect presence of a natural infection. Others were kept as nonexposed controls to check on suitability of water quality and were also examined for infection at the termination of the experiment.

Groups of each species were exposed to live snails for three or five days in either 70-gallon aquaria or two-gallon containers provided with a constant flow of cercaria-free well water at 10 C. The

experiments were conducted in a 15-17 C constant temperature room. Fish were killed from 1 to 106 days after exposure and examined for encysted metacercariae. Cysts were administered to hamsters in the previously stated manner.

Snails used in these experiments were obtained from Oak Creek and the Yaquina River in western Oregon. Development of cercariae was incomplete in snails collected during the months of December through March. Maturation of the cercariae was accomplished by holding snails at 20-22 C for 15 days. The incidence of infection in the snails used was determined by dissection of some individuals before the experiment and dissection of the remaining ones at the end of the exposure period.

Daily temperatures of the water were recorded, and in one experiment daily dissolved oxygen and pH determinations were made.

IV. Metacercarial Development in Freshwater Animals

Measurements of encysted metacercariae dissected from the external surface or from the kidneys of naturally infected Pacific giant salamanders, brook lampreys, Pacific lampreys, sculpins, redside shiners, coho salmon, and from experimentally infected steelhead trout sac-fry, Atlantic salmon, rainbow trout, lake trout, sculpin and mosquitofish, were made with an ocular micrometer. These are the same animals referred to in Section III. At least five

cysts each from naturally infected species were measured at various times from 1 to 106 days after exposure of the fish to infected snails. The measurements comprised minimum and maximum diameters of the cyst including the cyst wall. The length of the stylet and diameter of the excretory bladder were also measured.

RESULTS

I. Distribution of O. silicula in Three Coastal Rivers in Oregon and Seasonal Incidence of Mature N. salmincola Infection in These and in Snails from an Inland Stream

Infected snails were found in both fresh and saline waters in the Alsea, Siletz, and Yaquina Rivers during all seasons of the year (Tables 1-3). The farthest points downstream at which snails were found were at sites 3, 4, and 7 (see Figures 1-3 for site location) on the Alsea, Siletz, and Yaquina Rivers, respectively. The maximum and minimum salinities, recorded during the study period, at these sites were: 4.2 parts per thousand (ppt) in August 1964 to 0.8 ppt in February 1965 in the Alsea; from 4.2 ppt in August 1964 to 0.4 ppt in February 1965 in the Siletz; and from 11.2 ppt in August 1964 to 4.2 ppt in February 1965 in the Yaquina.

A total of 1695 snails was collected from the three coastal rivers during all seasons of the year. A total of 643 of the above snails had aperture diameters from 5.0 to 11.5 mm. Incidences of infection ranged from 52 (November 1964) to 9 (February 1965) percent in snails from the Alsea River; 40 (December 1964) to 9 (April 1965) percent in snails from the Siletz River; and 57 (August 1964) to 11 (May 1965) percent in snails from the Yaquina River (Tables 1-3). However, these differences may not indicate a true seasonal

Table 1. Monthly incidence of N. salmincola infection in snails in the Alsea River, Oregon.

Date	Number examined	Number infected				Percent infected			Ave. Temp. °C	Maximum salinity[3]
		N. salmincola		Other spp.	Total	N. salmincola		Total		
		Imm.	Mature			Imm.	Mature			
8/5/64	36	0	17	16	33[1]	0	47	47	20	4.2
8/21/64	150	0	22	44	66[2]	0	15	15	20	4.2
9/11/64	30	0	15	7	22	0	50	50	17	3.9
10/14/64	30	0	15	7	22	0	50	50	16	3.0
11/12/64	25	5	8	3	16	20	32	52	12	3.0
12/4/64	20	9	1	2	12	45	5	50	10	2.0
2/20/65	64	6	0	15	21	9	0	9	5	0.8
3/7/65	10	1	0	6	7	10	0	10	6	2.0
4/6/65	50	2	9	15	26	4	18	22	10	3.0
5/7/65	20	0	7	2	9	0	35	35	12	4.1
Totals	435	23	94	117	234			26.9		

[1] 3 with a mixed infection

[2] 4 with a mixed infection

[3] Recorded at site 3 (Figure 1)

Table 2. Monthly incidence of N. salmincola infection in snails in the Siletz River, Oregon.

Date	Number examined	Number infected				Percent infected			Ave. Temp. °C	Maximum salinity[2]
		N. salmincola		Other spp.	Total	N. salmincola		Total		
		Imm.	Mature			Imm.	Mature			
8/20/64	50	0	18	12	30	0	36	36	23	4.2
9/12/64	150	0	49	45	94[1]	9	32	32	20	3.9
10/13/64	30	0	9	11	20	0	33	33	17	3.0
11/12/64	30	2	8	7	17	7	27	34	12	2.2
12/5/64	21	8	0	3	11	40	0	40	10	1.5
2/10/65	15	2	0	5	7	13	0	13	7	0.4
3/12/65	25	5	0	10	15	20	0	20	8	0.8
4/2/65	42	4	0	23	27	9	0	9	9	2.0
5/2/65	25	3	3	11	17	12	12	24	11	3.8
Totals	388	24	87	127	238			28.6		

[1] 4 with a mixed infection

[2] Recorded at site 4 (Figure 2)

Table 3. Monthly incidence of N. salmincola infection in snails in the Yaquina River, Oregon

Date	Number examined	Number infected				Percent infected			Ave. Temp. °C	Maximum salinity[3]
		N. salmincola		spp.	Total	N. salmincola		Total		
		Imm.	Mature			Imm.	Mature			
8/5/64	30	0	17	13	30[1]	0	57	57	22	10.1
8/10/64	325	0	75	112	187[2]	0	20	20	22	11.2
8/21/64	30	0	12	3	15	0	40	40	20	10.2
9/12/64	30	0	13	5	18	0	43	43	18	9.8
10/15/64	25	0	9	4	13	0	36	36	15	9.0
11/13/64	25	3	8	3	14	12	32	44	12	8.2
12/8/64	15	3	5	3	11	20	33	53	9	---
1/29/65	5	0	0	0	0	0	0	0	8	---
2/1/65	30	14	0	6	20	47	0	47	7	---
2/2/65	59	25	0	11	36	43	0	43	6	4.2
2/10/65	145	27	0	4	31	18	0	18	6	---
2/20/65	25	10	0	8	18	40	0	40	7	5.4
3/2/65	25	11	0	3	15	44	0	44	9	7.1
4/8/65	25	2	7	1	10	8	28	36	10	8.4
4/20/65	25	2	8	2	12	8	32	40	12	9.0
5/7/65	53	2	4	6	12	4	8	11	15	10.2
Totals	872	99	158	184	441			29.5		

[1] 2 with a mixed infection

[2] 2 with a mixed infection

[3] Recorded at site 7 (Figure 3)

incidence of infection in snails, since sample sizes in some collections were small. The total percent infected snails from the Alsea, Siletz, and Yaquina Rivers was 26.9, 28.6, and 29.5, respectively. Fifteen snails were found to have mixed infections consisting of N. salmincola and a longtailed cercariae, and in some cases two other species of cercariae. These other species were described by Burns and Pratt (1953), Knight and Pratt (1955) and Burns (1961). A total of 1530 snails, having aperture diameters from 6.0 to 13.0 mm, was collected from Oak Creek from 29 January to 16 June 1965 and 34.8 percent were found infected with N. salmincola (Table 4).

Nineteen percent, 37 percent, and 76 percent of all snails having aperture diameters from 5-8 mm, 8-10 mm, and 10-13 mm, respectively, were infected with N. salmincola (Figures 5-8). The smallest snails found infected had aperture diameters of 6 mm. Immature cercariae were first noted in snails from the three coastal rivers during the second week of November (Tables 1-3). Mature cercariae were no longer observed in snails after the first week of December 1964, and were not observed again until the first week of April 1965 (Figure 9). Average maximum and minimum temperatures recorded during the study periods were: Alsea, 20 C (August 1964) and 5 C (February 1965); Siletz, 23 C (August 1964) and 7 C (February 1965); Yaquina, 22 C (August 1964) and 6 C (February 1965).

Table 4. Weekly incidence of N. salmincola infection in snails from Oak Creek.

Date	Number examined	Number infected				Percent infected			Ave. Temp. °C
		N. salmincola				N. salmincola			
		Imm.	Mature	spp.	Total	Imm.	Mature	Total	
1/29/65	50	26	0	4	30	52	0	52	6.7
2/1/65	51	21	0	14	35	41	0	41	8.0
2/8/65	10	5	0	0	5	50	0	50	7.5
2/10/65	20	11	0	1	12	55	0	55	8.0
2/15/65	20	6	0	2	8	30	0	30	8.5
2/26/65	7	5	0	0	5	71	0	71	9.0
3/1/65	159	39	0	22	61	24	0	24	9.0
3/3/65	415	89	0	32	121	21	0	21	10.0
3/8/65	17	8	0	2	5	17	0	17	9.5
3/12/65	15	5	0	2	7	33	0	33	9.0
3/18/65	30	6	0	4	10	20	0	20	7.0
3/21/65	15	1	0	1	2	7	0	7	9.0
3/30/65	22	9	0	1	10	41	0	41	10.5
4/1/65	39	1	1	4	4	3	3	5	9.0
4/8/65	50	13	12	3	28	26	24	50	10.0
4/12/65	255	21	78	16	115	8	35	43	10.0
4/22/65	25	1	10	3	14	4	40	44	10.5
4/29/65	180	4	101	25	131	2	59	61	11.5
5/6/65	25	0	7	2	9	0	24	24	12.0
5/13/65	25	0	9	1	10	0	36	36	12.0
5/20/65	25	0	9	2	11	0	36	36	12.0
5/27/65	25	0	9	3	12	0	36	36	13.0
6/16/65	50	0	30	15	45	0	60	60	16.0
Totals	1530	266	266	159	690			34.8	

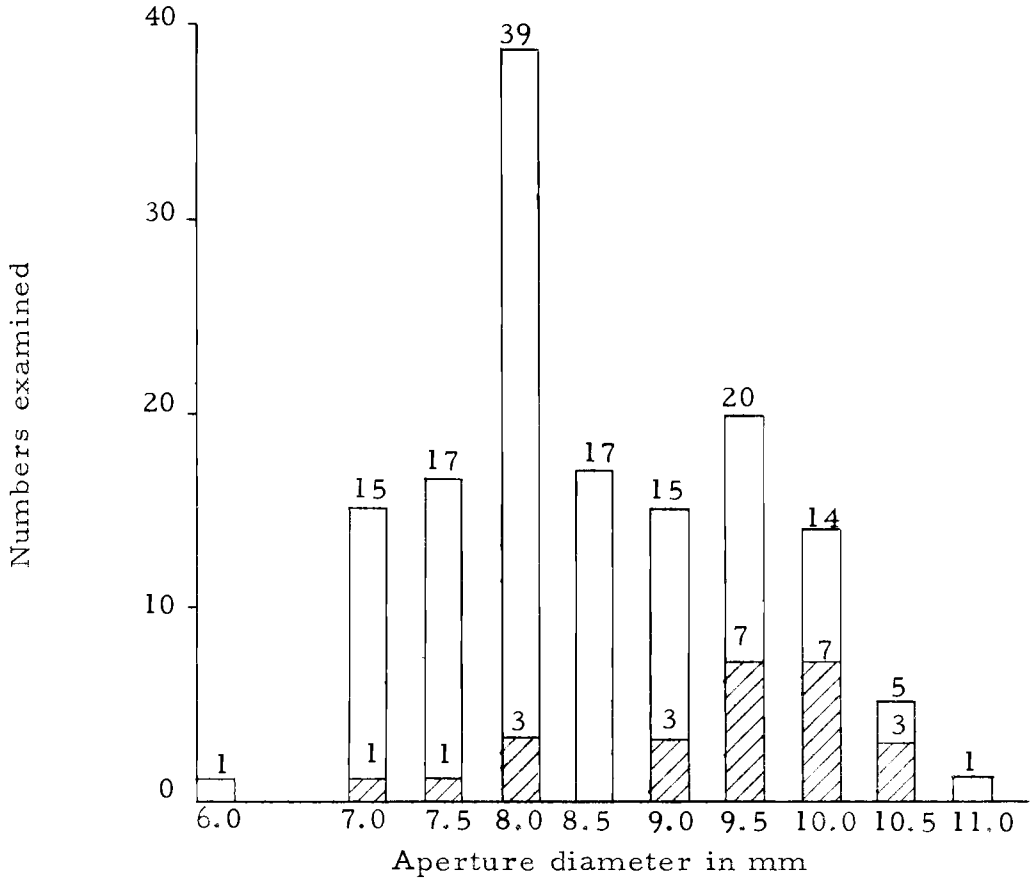


Figure 5. Size of snails and incidence of infection in snails collected from the Alsea River from 20 February 1965 to 7 May 1965. The shaded area represents infected snails.

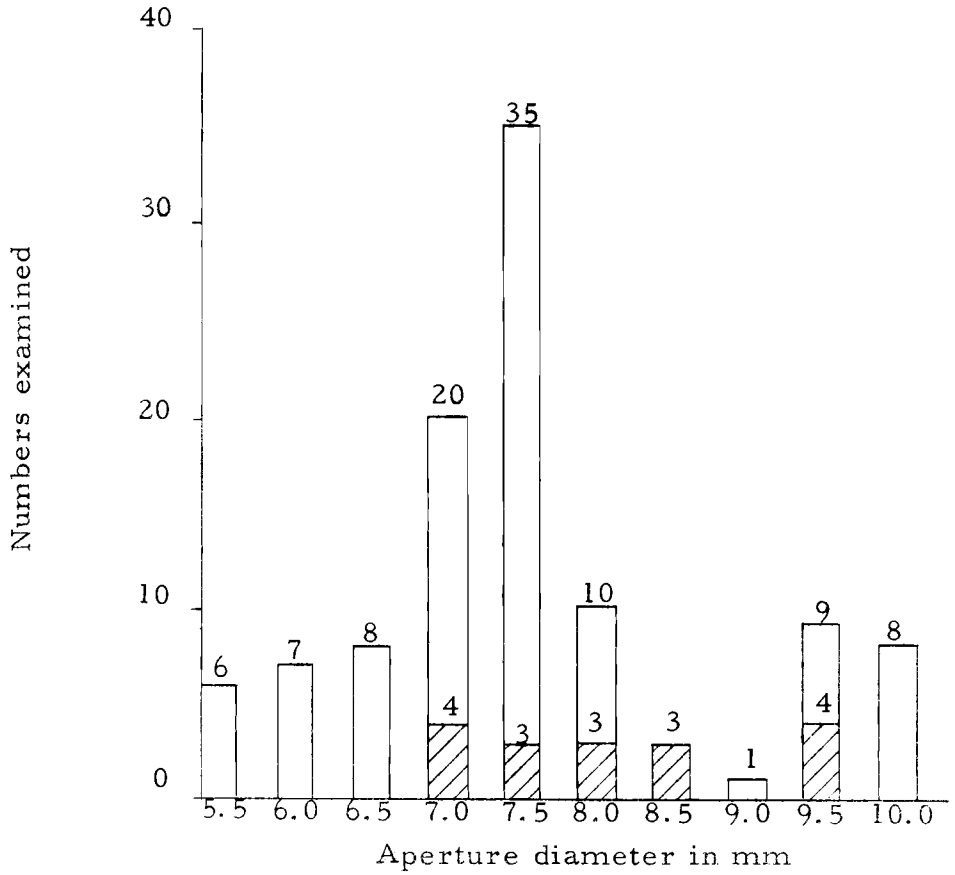


Figure 6. Size of snails and incidence of infection in snails collected from the Siletz River from 10 February 1965 to 2 May 1965. The shaded area represents infected snails.

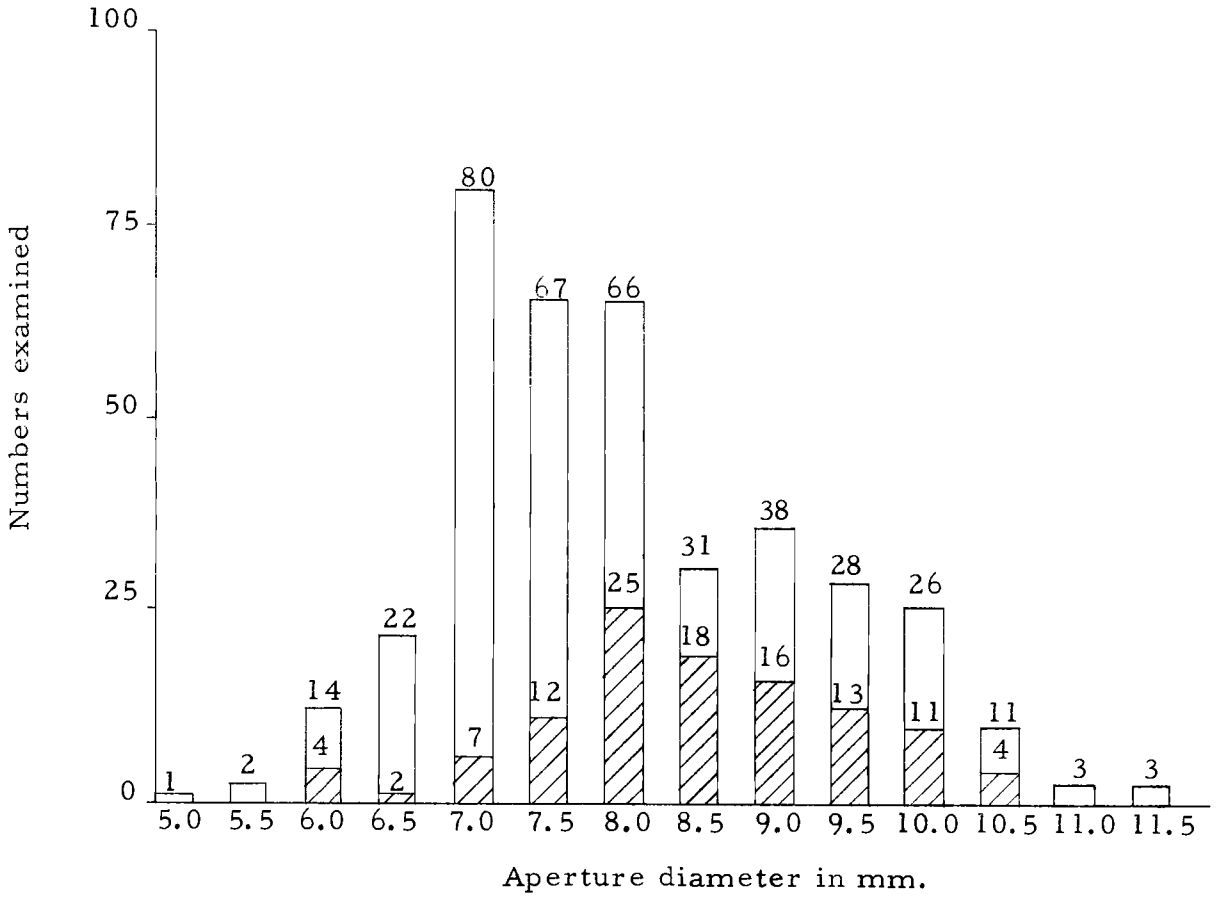


Figure 7. Size of snails and incidence of infection in snails collected from the Yaquina River from 29 January 1965 to 7 May 1965. The shaded area represents infected snails.

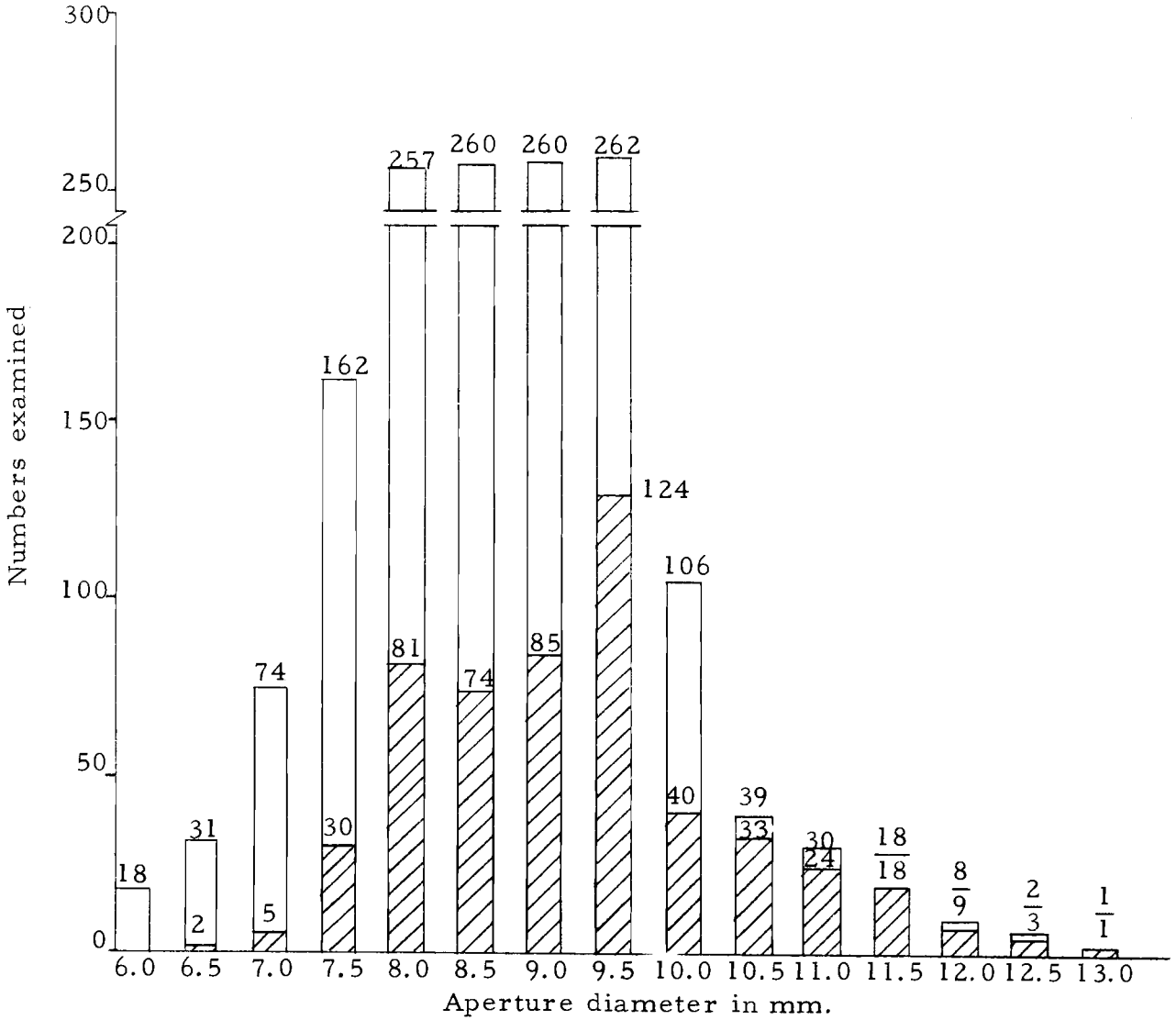


Figure 8. Size of snails and incidence of infection in snails collected from Oak Creek from 29 January 1965 to 16 June 1965. The shaded area represents infected snails.

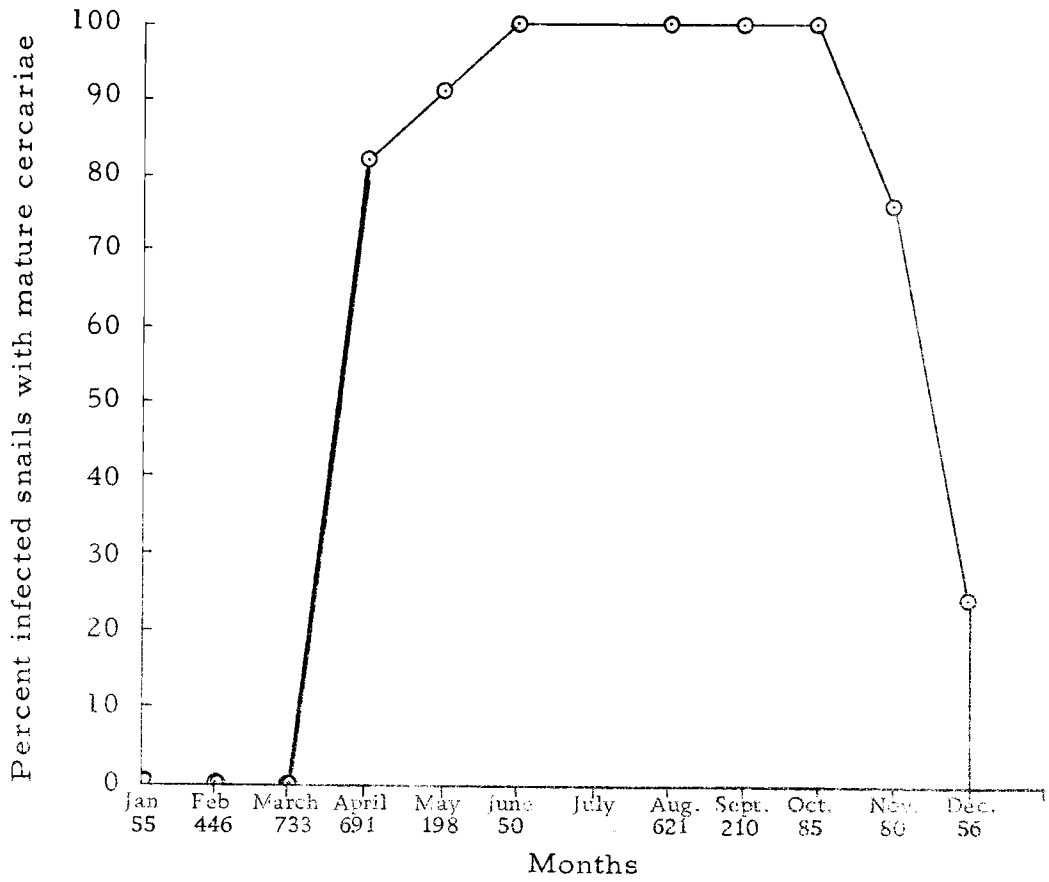


Figure 9. Monthly incidence of mature cercariae in infected snails (summary of data presented in Tables 1-4). The number below each month is the number of snails examined during that month.

II. Development of Cercariae of N. salmincola in Snails under Natural and Experimental Conditions

Infections were recorded as immature or mature based on the morphology and motility of the cercariae. Motile cercariae which varied in length from 310 to 470 μ and in width from 30 to 150 μ were considered mature (Figure 11). Nonmotile cercariae which varied in length from 115 to 156 μ and width from 76 to 96 μ and with little or no internal differentiation were considered to be immature (Figure 12). Four stages in the maturation of the cercariae were: (I) immature, as described above and shown in Figure 12; (II) immature, nonmotile, but slightly more elongate and narrower than (I). Internal structures not well defined; (III) immature, nonmotile, but is the same size and much like mature cercariae in that suckers, stylets and excretory bladders are well defined. Some may contract very slightly; (IV) mature, as described above and shown in Figure 11.

Natural Conditions

The results on the time of appearance of immature cercariae (Stage I) and the time of disappearance of mature cercariae (Stage IV) in snails were presented in the previous section. Identity of the immature cercariae as N. salmincola in samples of these snails was confirmed by the results of the experiment described below.

Experimental Conditions

Results of the experiments on maturation of cercariae in 600 snails held at room temperature (20-22 C) are shown in Figure 10. Mature cercariae (Stage IV) of N. salmincola were first noted in the snails after seven days. On day 15, 90 percent of the snails had some mature (Stage IV) cercariae.

Results of the experiment on cercarial maturation in snails held at 10, 16, and 22 C are presented in Table 5. Mature cercariae first appeared in snails held at the two lower temperatures after 11 to 15 days, and in snails at 22 C after six to ten days.

III. Natural and Experimental Infections of Marine and Freshwater Animals

Natural Infections

Marine Fish

The results of this study have been published (Millemann, Gebhardt, and Knapp, 1964), and are briefly summarized here. Eleven of fifteen ocean-caught chinook salmon, two to five years old, were found infected with metacercariae of N. salmincola. The number of cysts in the kidneys ranged from 7 to 70. One-hundred-sixteen of 152 ocean-caught coho salmon, two to three years old, were also found infected (Figure 13). The number of cysts per

Table 5. Effect of temperature on maturation of cercariae in snails.

Time in days	Water °C	Number examined	Number infected			Percent infected		
			Imm.	Mature	Total	Imm.	Mature	Total
5	10	5	2	0	2	40	0	40
10		25	5	0	5	20	0	20
12		25	5	0	5	20	0	20
15		25	2	4	6	8	16	24
18		25	2	4	6	8	16	24
21		25	0	6	6	0	24	24
5	16	5	1	0	1	20	0	20
10		25	4	0	4	16	0	16
12		25	2	4	6	8	16	24
15		25	3	0	3	12	0	12
18		25	0	6	6	0	24	24
21		25	0	0	0	0	0	0
5	22	5	1	0	1	20	0	20
10		25	4	2	6	16	8	24
12		25	4	3	7	16	12	28
15		25	0	5	5	0	20	20
18		25	0	7	7	0	28	28
21		25	1	6	7	4	24	28
Totals		390	36	47	83			21

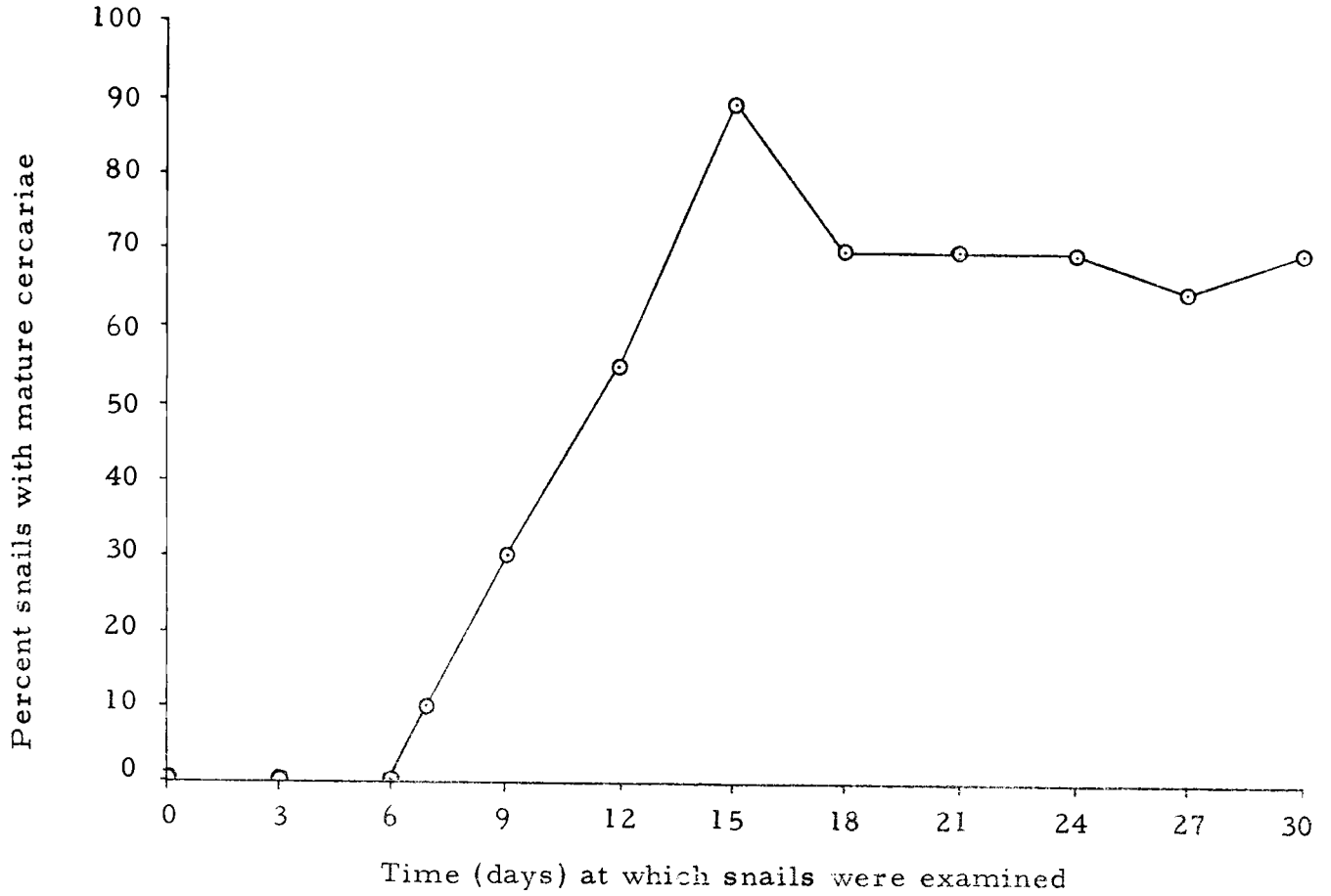


Figure 10. Results of three experiments on maturation of N. salmincola cercariae in 600 snails collected from the Alsea, Siletz and Yaquina Rivers and held at room temperature (20-22 C) from 29 January to 1 March 1965.

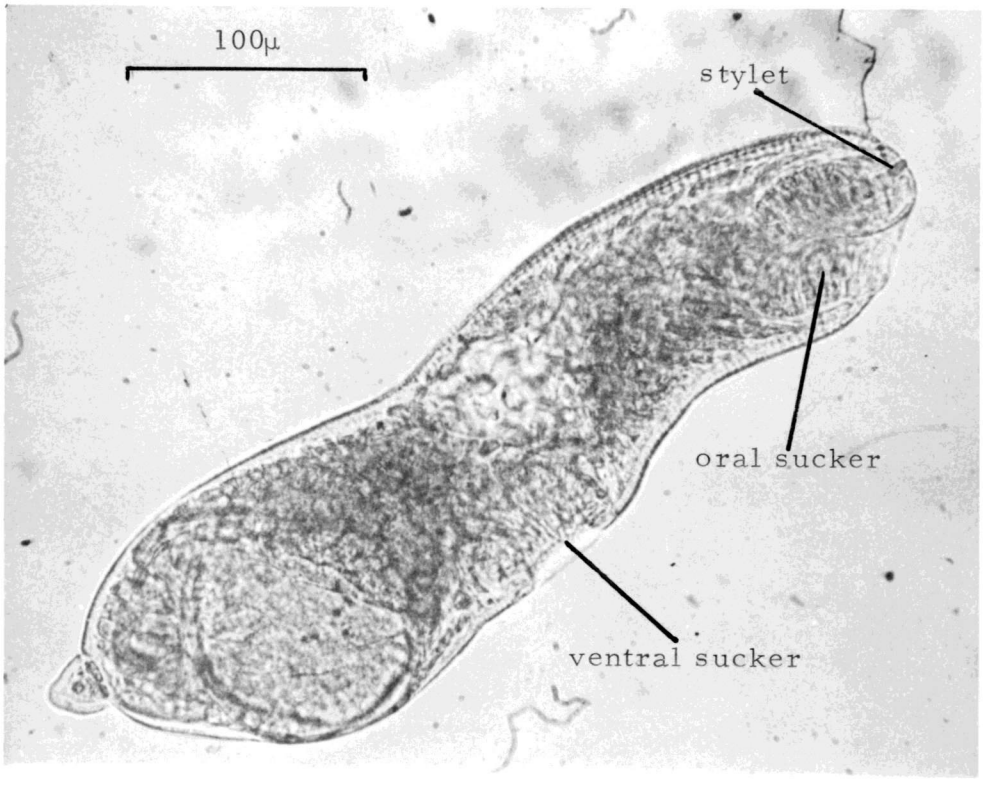


Figure 11. Mature cercariae (stage IV) of N. salmincola obtained from snails during the months of April through November.

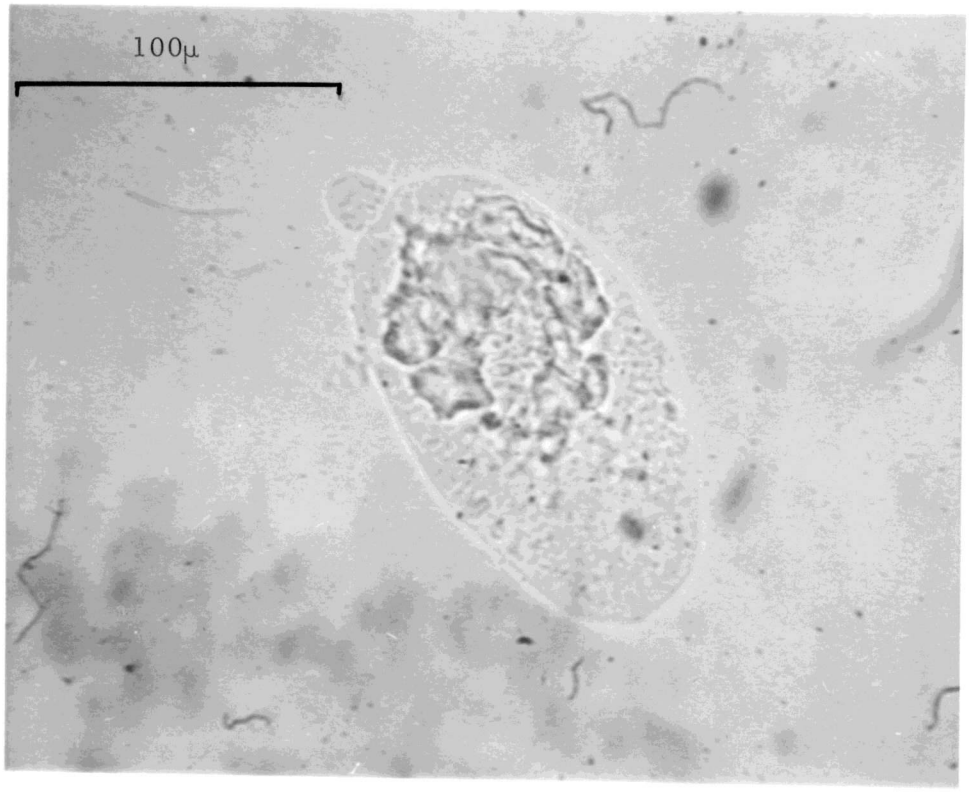


Figure 12. Immature cercariae (stage I) of N. salmincola obtained from snails during the months of December through March.

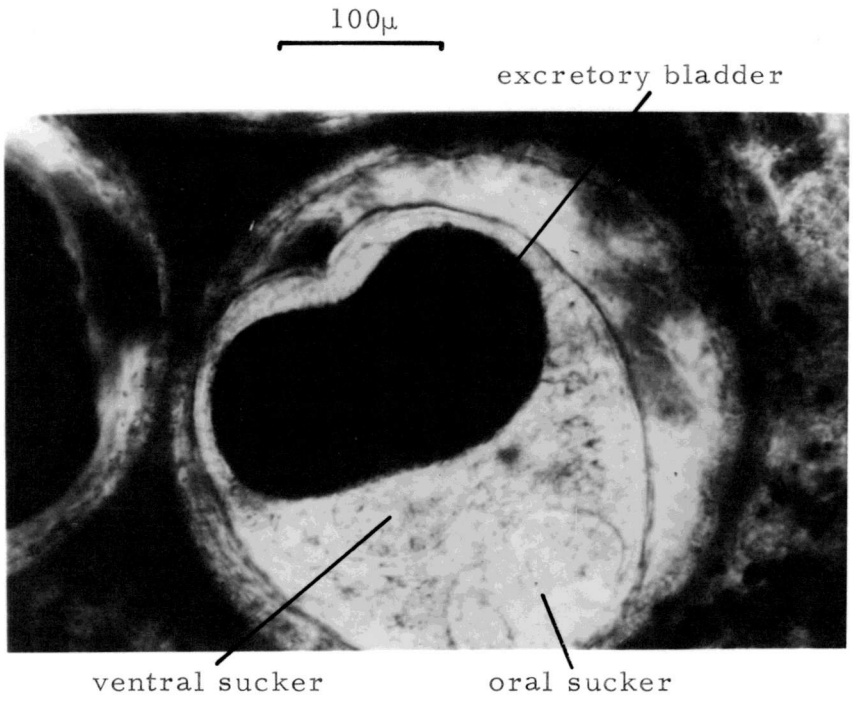


Figure 13. Metacercariae from the kidney of an ocean-caught infected coho salmon collected August 6, 1963.

kidney ranged from 1 to 2400. Additional unpublished data concerning weights, lengths, sex, and number of metacercariae per kidney of individual fish are presented in Table 10. The identification of the parasites as N. salmincola was confirmed by the recovery of adult flukes from a dog fed infected kidneys from two coho salmon. This dog also developed typical "salmon poisoning" disease which proved that the rickettsiae had remained infectious in fish which had been in the ocean for up to 18 months.

The incidence of infection in ocean-caught salmon increased throughout the summer of 1963. Salmon collected early in the summer probably originated outside the endemic area because salmon are known to migrate long distances during their residence in the ocean.

Metacercariae were not found in the kidneys of three pink salmon.

Freshwater Animals

The Pacific giant salamander and four species of nonsalmonid fishes representing three families were found naturally infected (Table 6). All other species of nonsalmonids (refer to Table 7), and 100 eulachon were found to be uninfected. Cysts were not seen on the external surfaces of the sculpin, redside shiner, brook lamprey, and Pacific lamprey. The parasites in the salamander were found only on the gills. The average number of cysts per animal of a given

species ranged from 4 (sculpin) to 50 (brook lamprey). The morphology of the encysted metacercariae conformed to that of N. salmincola. The identification of the parasites as N. salmincola was confirmed by the recovery of adult flukes from hamsters given cysts from the salamander, sculpin, redside shiner, and brook lamprey. No adults were found in a hamster given cysts from the Pacific lamprey possibly because of the small number, approximately 100, of cysts administered.

Experimental Infections

In five experiments, 14 species of fishes representing eight families were experimentally infected (Table 7). All pre-exposure (fish examined for infection before the start of the experiment) and nonexposed controls were negative except for the redside shiner, sculpin, and brook lamprey, which were naturally infected. Unfortunately, uninfected fish of these last species, which could have been obtained outside the enzootic area, were not used in these experiments. However, it is likely that an experimental infection was established in these fishes. The number of cysts recovered from the fish comprising the experimental groups was greater than in the unexposed groups (for redside shiners, sculpins, and brook lampreys, averages of 30, 20, and 150 cysts per experimentally exposed fish, 5, 4, and 50 cysts per naturally exposed fish). Moreover, many of these cysts were of recent origin, since they were smaller, the cyst

Table 6. Animals naturally infected with N. salmincola.

Host	Number infected/Number examined	Percent infected
Ambystomidae		
Dicamptodon ensatus	14/14	100
Petromyzontidae		
Lampetra richardsoni	28/28	100
L. tridentata	5/5	100
Cottidae		
Cottus perplexus	69/74	93
Cyprinidae		
Richardsonius balteatus	30/67	45

Table 7. Results of experimental exposure of fishes to N. salmincola.

Host	Total pre-exposure & non-exposured controls		Exposed			Snails	
	Number examined	Number infected	Number	Number infected	Percent infected	Number used	Percent infected
Salmonidae							
Salmo gairdneri							
rainbow[1]	25	0	10	10	100	70	20
steelhead sac-fry[1]	75	0	50	50	100	57	50
S. salar[1]	36	0	30	25	83	96	17
S. trutta[1]	50	0	50	50	100	50	52
Salvelinus fontinalis[1]	50	0	50	50	100	50	68
S. namaycush[1]	21	0	25	25	100	96	31
Petromyzontidae							
Lampetra richardsoni	9	9	6	6	100	25	52
Cottidae							
Cottus perplexus	22	22	25	25	100	33	38
Cyprinidae							
Carassius auratus	25	0	21	21	100	30	67
Richardsonius balteatus	27	5	20	20	100	33	35
Catostomidae							
Catostomus macrocheilus	8	0	5	5	100	33	33
Centrarchidae							
Lepomis macrochirus	25	0	23	23	100	30	60
Gasterosteidae							
Gasterosteus a. aculeatus	40	0	25	25	100	33	36
G. a. microcephalus	25	0	25	25	100	30	47
Poeciliidae							
Gambusia affinis[1]	50	0	50	50	100	58	50
Percidae							
Perca flavescens	15	0	25	0	0	30	44

[1] Combined results of two experiments.

walls were thinner, and the excretory bladders were not filled with the characteristic granules seen in older cysts. Finally, all the redside shiners were infected after experimental exposure, whereas only 19 percent of the controls were naturally infected. The average number of cysts per animal of a given species ranged from 20 (sculpin) to 278 (goldfish). Cysts from all species except rainbow and steelhead trout and the brook lamprey were given to hamsters. In all instances adult flukes were subsequently recovered from the hamsters. The morphology of the encysted metacercariae agreed with that of N. salmincola.

As first noted by Bennington and Pratt (1960), the parasites in experimentally infected nonsalmonids tended to concentrate in the superficial tissues, including gills and fins, more than in the visceral organs. I also observed this distribution in naturally infected nonsalmonid fish.

In one experiment, all of 25 steelhead sac-fry, 25 brook trout, and 25 brown trout died during the first three days of exposure to the snails. The temperature, pH, and dissolved oxygen concentration of the water in which these fish were held was, respectively, 13.5 C, 8.02, and 7.2-9.2 mg/l. The incidence and intensity of infection in these snails was considerably greater than in snails used in other experiments. The fins of the fishes were badly eroded, and the body surfaces were almost completely covered with cysts. The

average number of cysts per fish was much greater in this experiment than in other experiments in which the fish survived exposure to snails with lighter infections. These results indicate that the parasites were lethal under the conditions of this experiment since temperature, pH, and dissolved oxygen were not unfavorable for the fish.

IV. Metacercarial Development in Freshwater Animals

Experimental Infections

The size of the cyst, and excretory bladder and thickness of the cyst wall increased with age (Table 8; Figures 14-15). Sizes (in microns) of encysted metacercariae from experimentally infected Atlantic salmon, and their approximate ages in days (in parenthesis) were: 155 x 158 to 181 x 188 (15); 188 x 188 to 204 x 211 (30); 208 x 219 to 248 x 252 (50); 227 x 231 to 242 x 248 (106). In older metacercariae the stylet was absent and the excretory bladder was filled with granules (Table 8; Figure 16). These observations are in agreement with those of Bennington and Pratt (1960).

Encysted metacercariae up to 24-hours-old from the caudal fins of steelhead trout sac-fry were similar in appearance to the young cysts described by Simms, et al. (1931) and Bennington (1951). The excretory bladder was empty, the cyst wall was thin, transparent

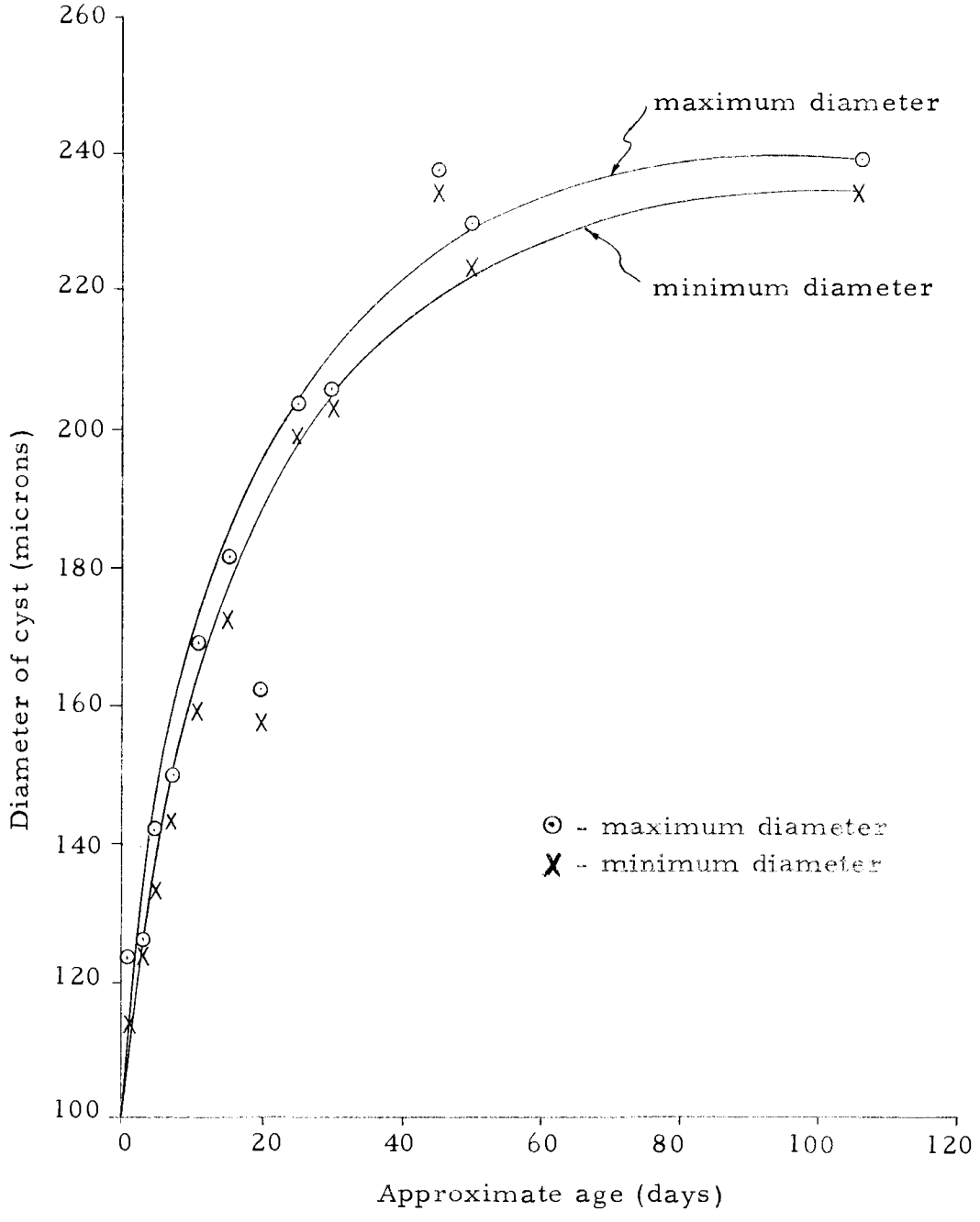


Figure 14. Increase in diameter of cysts, obtained from experimentally infected fishes, with age.

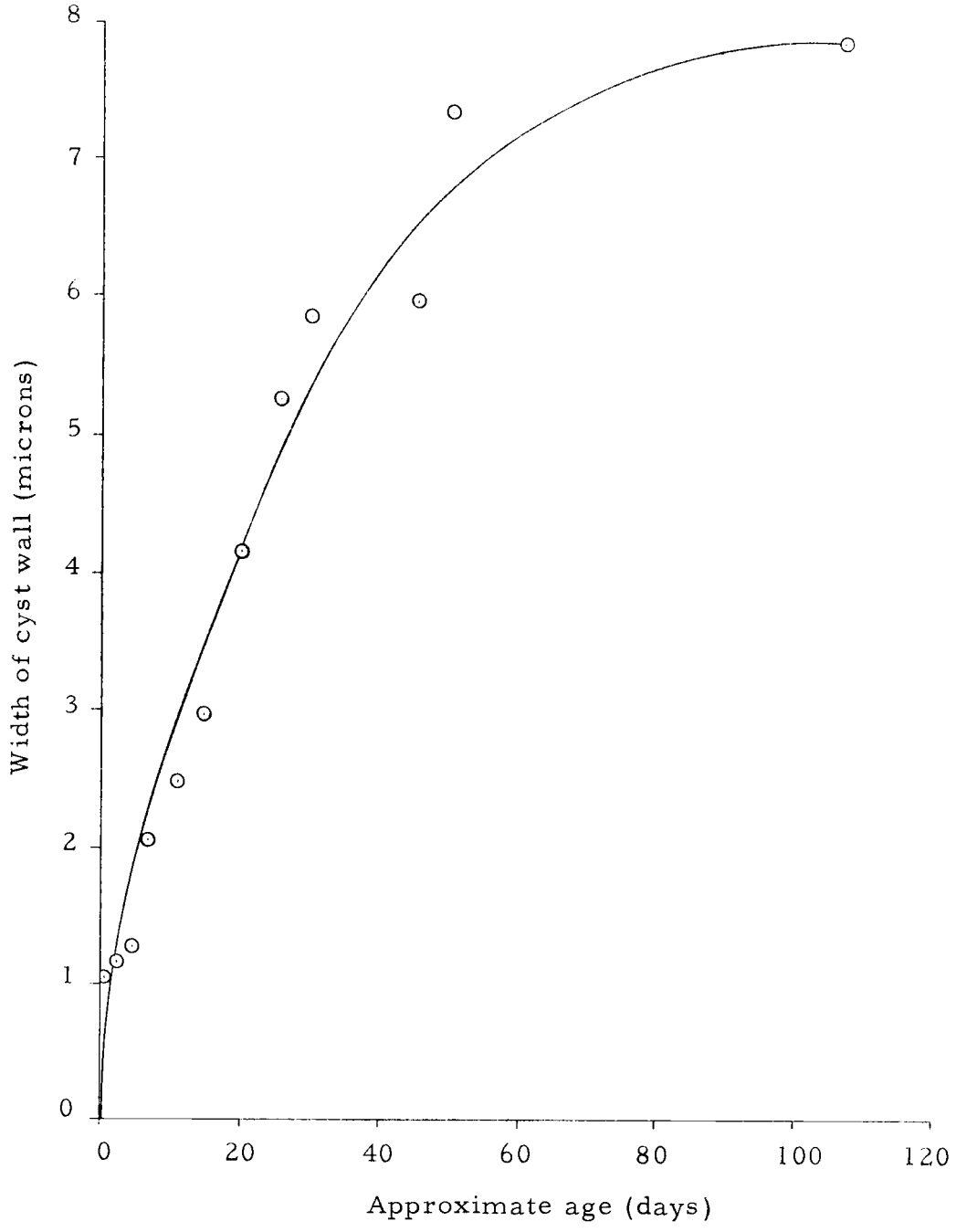


Figure 15. Increase in width of cyst wall with age. Cysts obtained from experimentally infected fishes.

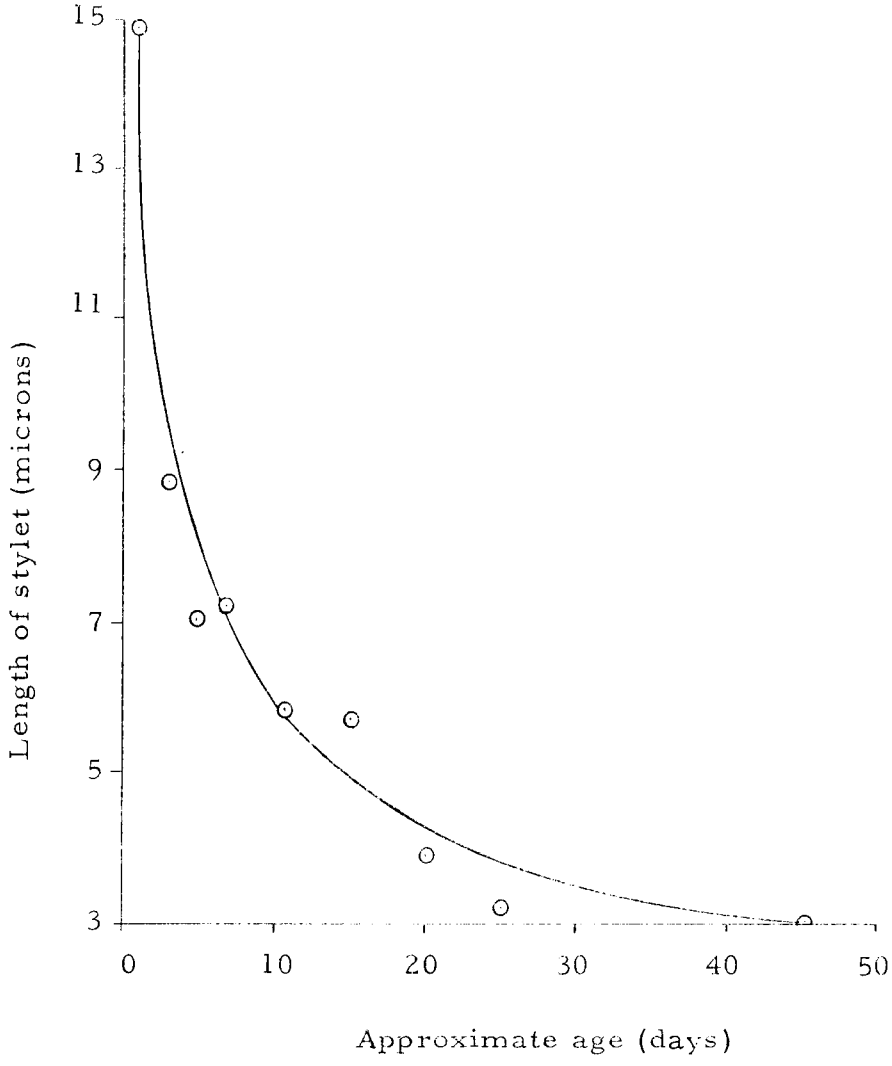


Figure 16. Average decrease in length of stylet with age. Cysts obtained from experimentally infected fishes.

and easily ruptured, and the stylet was present (Figure 17). These cysts were clear and not as readily seen as older cysts which were opaque and appeared as white spots in fresh fish tissue. Very young metacercariae occupied approximately two-thirds of the volume of the cyst and were extremely active, whereas, older metacercariae were sluggish and nearly filled the cyst.

Measurements of older cysts agreed with those reported by Donham, Simms and Miller (1926) and Simms et al. (1931). No significant differences were noted in the morphology and size of cysts of approximately the same age from different hosts.

Natural Infections

Measurements of five encysted metacercariae from each of five species of naturally infected animals are presented in Table 9. These agree with the measurements given by Donham, Simms and Miller (1926) for encysted metacercariae of N. salmincola obtained from naturally infected salmonid fish.

Ten cysts which were dissected from the muscles at the base of the caudal and pectoral fins and from the head of the sculpin had cyst walls that were not well-defined and consisted of what appeared to be fibrous connective tissue with widths varying from 3.3 to 8.0 μ in the smallest cyst and 23.1 to 39.6 μ in the largest cyst (Figure 18). The cysts, including the concentric, undefined cyst wall, measured

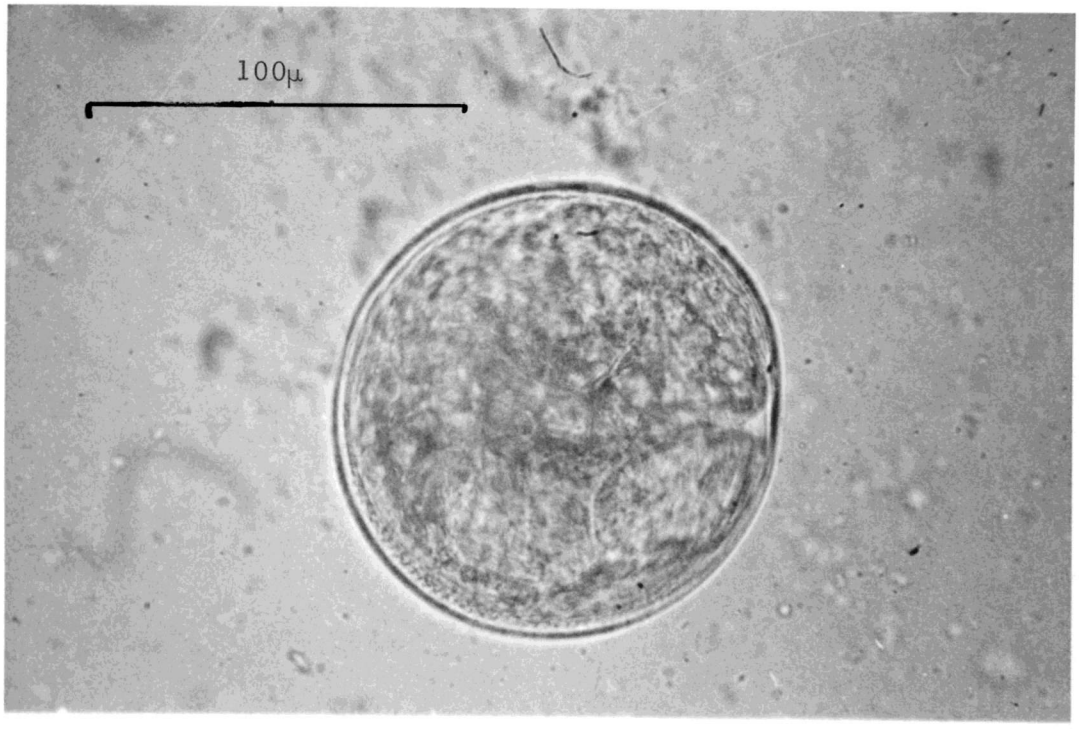


Figure 17. Approximately 24-hour-old encysted metacercariae dissected from the skin of steelhead trout sac-fry.

Table 8. Measurements (in microns) of metacercarial cysts from naturally infected animals.

Host species	Cyst dia. Max.	Cyst dia. Min.	Thickness cyst wall	Length of stylet	Excr. bladder Max.	Excr. bladder Min.
Salamander	221	208	7.0	---[1]	112	53
	198	195	3.3	---	102	69
	333	289	9.0	---	234	96
	252	250	8.0	---	128	72
	282	275	8.0	---	135	80
River lamprey	178	172	3.0	4.0	106	50
	228	165	4.0	3.0	115	69
	198	149	3.0	---	99	46
	192	188	3.3	---	112	69
	204	201	4.0	---	110	73
Brook lamprey	193	189	3.0	4.0	108	91
	201	195	4.0	---	115	68
	190	181	4.0	---	133	69
	202	193	4.0	---	122	83
	210	208	4.0	---	133	90
Redside shiner	282	278	8.0	---	142	101
	198	189	4.0	---	100	62
	293	290	8.5	---	139	90
	272	269	8.0	---	150	100
	201	200	4.0	---	141	72
Reticulate sculpin	215	182	---[2]	---	129	125
	204	191	---	---	109	50
	196	165	---	---	99	79
	210	199	---	---	100	43
	211	198	---	---	142	99
	185	165	---	---	92	63
	205	201	---	---	116	76
	271	248	---	---	116	89
	281	264	---	---	122	82
	298	271	---	---	92	50

[1] Stylet absent

[2] Cyst wall not well defined

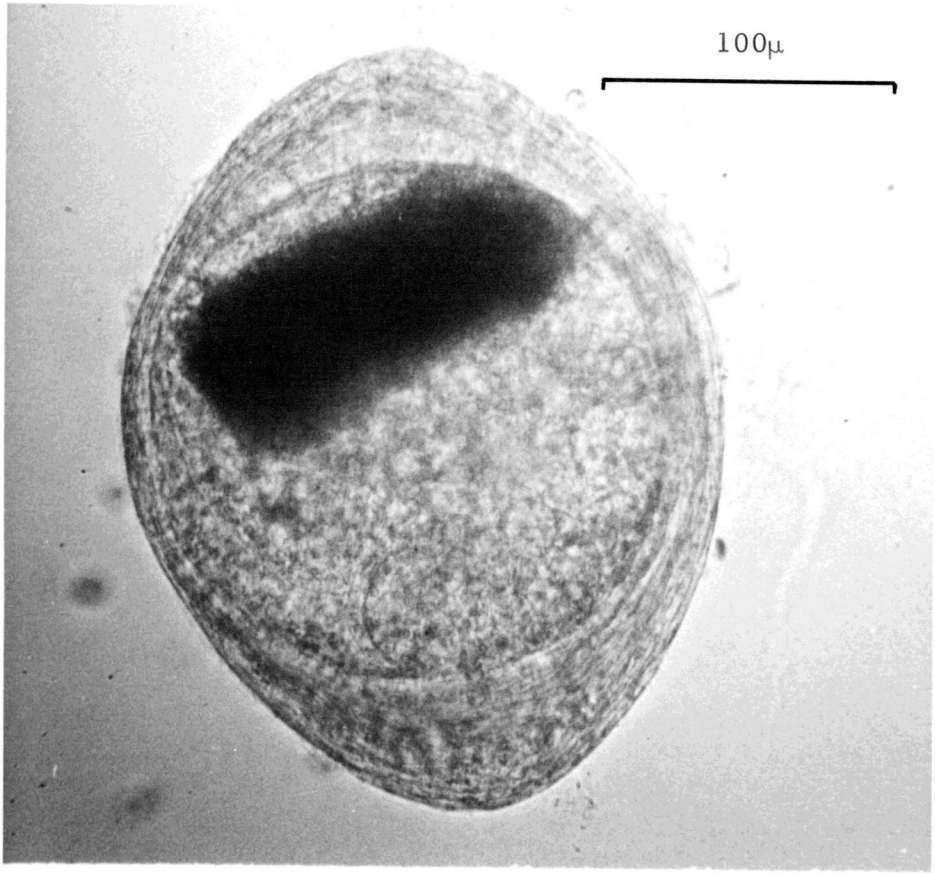


Figure 18. Metacercarial cyst dissected from the muscles of a naturally infected reticulate sculpin.

165.0 x 185.0 μ to 271.0 x 298.0 μ. The stylet was absent in all ten cysts.

DISCUSSION

The snail, O. silicula, is widely distributed throughout the Pacific northwest in the rivers and streams located in the enzootic area of "salmon poisoning" disease. The results of my studies constitute the first report of the occurrence of O. silicula in brackish water. The absence of young snails in saline waters during the time when large numbers of them are present in freshwater in the same stream suggests that they do not reproduce in the former environment. Snails may be carried downstream each year during periods of flooding, and because of their ability to survive in waters with low salinities, are able to adapt and establish themselves in the new environment. Snails which occurred in brackish water were not larger than 10.0 mm in aperture diameter and were generally smaller than snails in freshwater in the same stream; however, the incidence of infection with N. salmincola was approximately the same in both groups. The reason for the absence of snails larger than 10.0 mm in aperture diameter, in saline waters, is not known. There is no information on the salinity tolerances of the miracidia and cercariae of N. salmincola. Thus, whether snails and fish can be infected in brackish water is not known.

This study showed a seasonal incidence of mature infections in snails. Mature cercariae were present in snails in late spring,

summer, and fall, whereas only immature cercariae were found in snails during the winter months. Bennington (1951) was also unable to find mature infections during the months of February and March. The knowledge that mature infections of cercariae of N. salmincola are not present in snails from the first week of December to the first week of April of the following year may be of practical use to hatcherymen and fish culturists in indicating the optimal time for release of hatchery fish into local streams, because at this time it would be unlikely that the fish would become infected.

The results of experiments on the effects of temperature on maturation of cercariae in snails under laboratory conditions showed that development of the cercariae to maturity could be accomplished by holding infected snails with immature infections at room temperature for 15 days. The effect of temperature on the rate of parasite development in snails was shown in experiments where infected snails were held at 10, 16, and 22 C. The cercariae matured more rapidly in snails held at 22 C than in snails held at 16 and 10 C. These results together with the observations on seasonal incidence of mature infections in snails under natural conditions, suggest that temperature may be an important factor in determining the rate of larval development in the snail host. Temperature has been shown

to affect directly the developmental rates of other species of trematodes in snails (Kingston, 1963; Stirewalt, 1954; Wagner and Moore, 1959). Photoperiod length may also affect the developmental rate of N. salmincola in snails.

Large snails, greater than 2.5 cm in total length (approximately 9.0 mm aperture diameter), which were infected with approximately ten different species of trematode cercariae, including N. salmincola, were found during all seasons of the year. These results are not in agreement with those of Simms, Donham and Shaw (1931), who found that snails of this size or greater were scarce in fall and winter, but plentiful in late spring and early summer. Probably the reason why these investigators were unable to find large snails during the winter and spring months was because the snails are sometimes covered with silt and are nearly always inaccessible during periods of high and cloudy water which may persist for several months. Bennington (1951), however, did collect large snails over 3.0 cm in total length (approximately 11-12 mm aperture diameter) during February and March from the Alsea River and Oak Creek.

This is the first report of mixed infections consisting of N. salmincola with one and sometimes two other species of trematode cercariae. In no instance, however, did snails with high numbers of N. salmincola harbor other trematode cercariae. Snails with mixed infections had low numbers of N. salmincola cercariae and

high numbers of one or two other species of other cercariae. Bennington (1951) and Bennington and Pratt (1960) did not find mixed infections. This may have been due to the small numbers of snails examined since in my studies mixed infections were found in only 15 of 4,215 snails. The "exclusion" by N. salmincola, when present in high numbers, of other trematodes from snails, if real, is interesting and deserves further study.

Bennington (1951), Simms, Donham and Shaw (1931) did not find snails less than 2 cm in total length infected with N. salmincola. I found infections in 71 of 571 snails with aperture diameters of 6.0 to 7.5 mm. The majority of snails with this aperture size are less than 2.0 cm in total length. The reason why the above investigators did not observe infections in very small snails was probably because of the low numbers examined.

This is the first report of the development of N. salmincola cercariae in snails. The immature cercariae, designated as stages I, II, and III, and considered here to be N. salmincola, may have represented another species of trematode. This is unlikely, however, because in snails held at room temperature, and having initially only immature cercariae, there was a gradual decrease in the numbers of immature stages (I, II and III) with a concomitant increase of mature cercariae (stage IV) with the passage of time. Definitive proof for the development of N. salmincola cercariae as described

here can only be shown by experimental infection of parasite-free snails with N. salmincola. Bennington (1951) stated that snails collected during the months of February and March were not infected with N. salmincola. He may not have recognized the immature cercariae and, therefore, many of his snails could have been infected with N. salmincola. I have observed only immature infections in snails during the above months.

This is the first report of natural infections in nonsalmonid fishes, and in an animal other than a fish, with the metacercariae of N. salmincola. The reason Donham, Simms and Miller (1926) and Simms et al. (1931) were unable to find natural infections in their fish was probably because of the small numbers examined.

It is not known if the parasites from the fish were carrying N. helminthoeca.

The failure to recover adult flukes from hamsters given cysts from naturally infected Pacific lampreys was probably due to the small number, approximately 100, administered. The minimal number of cysts needed to establish an infection in this animal has not been determined.

The results from the experimental infections agree with those of Bennington and Pratt (1960) since I was able to infect the sculpin and goldfish. Pre-infection controls for the sculpin, brook lamprey, and redside shiner showed that the experimental fish were naturally

infected. However, an experimental infection was probably established because the number of cysts recovered from the fish comprising the experimental groups was greater than that in the unexposed groups. Moreover, many of these cysts were of recent origin since they were smaller, the cyst wall thinner, and the excretory bladder not filled with the characteristic granules seen in older cysts. Finally, in the case of the redside shiner all the fish were infected after experimental exposure, whereas, only 19 percent of the controls were naturally infected.

These results extend the number of nonsalmonid fishes susceptible to experimental infection to include six new species in six familes. It is unlikely that pondfishes such as mosquitofish, goldfish, and bluegills would be naturally infected since the snail occurs predominantly in streams, unless the ponds received water from streams containing infected snails. The results on the experimental infection of the brook trout agree with those of Simms et al. (1931) who also infected this species. Infections of the brown trout, lake trout, and Atlantic salmon add three new species to the known number of susceptible salmonids.

It is apparent from these results that there is little host specificity shown by this parasite. It is not known how much of a role the naturally infected nonsalmonids, as opposed to salmonids, play in maintaining the life cycle of the parasite. There were fewer

parasites in all of the naturally infected non-salmonids than in native cutthroat trout, Salmo clarki, taken from the same stream. Final assessment of the role of non-salmonid fishes in maintaining natural infections must await information on their relative availability to the definitive host(s).

The results of the experiment in which the steelhead sac-fry, brook trout, and brown trout died during a three-day exposure to very large numbers of cercariae suggest that the parasites under these conditions were lethal since temperature, pH, and dissolved oxygen values were suitable.

This is the first report of descriptions of metacercariae of known age. Donham, Simms and Miller (1926) gave 140 μ as the approximate diameter of encysted metacercariae in fish. According to my results, these cysts were probably from one to five days old. Ward and Mueller (1926) observed cysts having diameters ranging from 166 to 246 μ in fish three to four months old. In view of the age of the fish and size of the cysts, the parasites were probably 15 to 106 days of age. Simms, et al. (1931) reported cysts having diameters ranging from 170 to 255 μ. Thus, these cysts were 11 days to nearly four months of age. It is apparent then, that differences in cyst sizes given by the earlier investigators can be explained on the basis of differences in the ages of the parasites. With additional information on the morphology and size of

metacercariae greater than 106 days of age, it would be possible to age parasites recovered from naturally infected fish and to determine the age at which the fish became infected.

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Appendix Table 1. Additional data concerning the numbers of metacercariae of N. salmincola in the kidneys of ocean-caught salmonids.

Species	Weight in pounds	Fork length in inches	Sex	Number of cysts per kidney
Silver	7.5	26.3	M	0
S	7.0	25.0	F	0
S	8.0	27.0	M	0
S	8.5	28.0	M	0
S	7.0	24.5	F	0
S	6.0	23.0	F	0
S	6.5	26.0	F	0
S	5.5	23.5	M	0
S	8.5	29.5	F	450
S	7.0	25.3	M	0
S	8.0	27.5	F	120
S	6.0	26.5	M	0
S	5.0	22.3	M	310
S	7.0	25.3	M	120
S	8.5	28.5	F	475
S	7.5	25.5	F	0
S	7.3	26.5	F	0
S	8.0	27.0	F	0
S	8.0	27.5	F	0
S	4.5	22.5	M	0
S	4.0	24.3	F	1230
S	5.8	25.0	M	0
S	6.5	26.0	M	81
Chinook	19.0	34.0	F	0
S	12.5	24.5	F	10
C	14.5	31.5	F	0
S	7.0	25.5	F	1100
S	6.0	25.0	M	0
S	7.5	26.3	F	480
S	7.5	26.0	M	480
S	8.5	27.5	M	30
C	4.5	21.5	M	20
S	8.0	27.0	M	90
S	7.5	25.5	F	390
S	10.0	28.0	M	0
S	8.5	28.0	M	25
S	7.5	25.5	F	170
S	5.0	22.5	F	1500

Appendix Table 1. Continued

Species	Weight in pounds	Fork length in inches	Sex	Number of cysts per kidney
S	7.5	25.3	M	475
S	5.5	23.5	M	1800
S	4.5	23.0	M	2000
S	6.5	25.5	F	1200
S	7.3	26.0	M	420
S	6.8	25.5	M	1350
S	7.5	25.5	F	220
S	5.5	24.0	M	2200
S	4.5	22.0	F	1750
S	4.0	21.0	M	1200
S	7.0	24.0	F	65
S	6.0	23.5	M	0
C	13.0	24.8	F	50
C	17.0	32.3	F	12
C	13.5	30.5	F	10
S	7.0	24.0	F	350
S	8.0	24.5	F	15
S	7.5	24.5	M	0
S	7.5	25.0	F	52
S	7.0	24.8	M	180
C	16.0	30.5	F	35
C	13.0	29.5	F	11
C	7.5	25.0	F	38
S	8.0	24.5	M	0
S	8.0	25.5	M	60
S	8.0	26.0	M	12
C	10.0	27.0	M	0
C	11.5	27.5	F	7
C	15.5	31.0	M	0
S	12.5	29.0	M	70
S	7.0	23.3	M	12
S	7.0	26.0	F	2400
C	7.5	25.0	F	20
C	12.5	30.0	F	9
S	7.5	25.0	F	14
S	6.5	22.0	F	80
S	6.5	22.5	M	0
S	7.0	22.0	M	12
S	7.0	24.0	M	36
S	10.0	27.0	M	84
S	7.5	25.0	M	120

Appendix Table 1. Continued

Species	Weight in pounds	Fork length in inches	Sex	Number of cysts per kidney
S	7.0	26.0	F	40
S	6.5	23.0	M	800
S	9.0	26.5	M	0
S	10.0	27.8	F	0
S	5.5	22.5	M	2
S	8.5	24.5	M	230
S	8.0	25.5	M	80
S	9.0	27.0	M	4
S	10.0	27.5	F	80
S	10.0	27.5	F	1
S	10.0	27.0	M	1
S	7.5	25.5	M	80
S	7.0	24.0	M	10
S	7.5	25.0	F	50
S	6.5	23.5	M	750
S	11.0	28.0	M	0
S	8.0	27.0	F	30
S	9.0	26.0	F	1
S	9.0	26.0	M	1
S	9.5	26.5	F	0
S	8.5	25.5	F	40
S	7.5	25.0	F	150
S	7.0	24.5	F	3
S	8.5	22.5	M	120
S	8.5	25.0	M	70
S	8.5	26.0	M	22
S	8.0	24.5	M	18
S	14.0	31.3	F	0
S	10.5	28.0	F	20
S	7.5	23.5	M	180
S	8.5	26.8	F	540
S	8.5	27.0	F	210
S	10.0	28.0	F	100
S	11.0	27.5	F	44
S	7.0	23.5	M	3
S	8.0	24.0	M	30
S	7.5	22.8	M	12
S	9.5	26.0	F	720
S	7.5	24.0	F	8
S	7.0	23.5	M	0
S	7.3	26.5	M	0

Appendix Table 1. Continued

Species	Weight in pounds	Fork length in inches	Sex	Number of cysts per kidney
S	7.5	25.5	F	70
S	6.5	23.5	F	30
S	8.5	28.5	M	150
S	7.3	26.3	F	230
S	8.0	26.5	F	185
S	7.8	25.0	F	120
S	7.0	26.3	F	30
S	6.3	24.5	M	29
S	7.5	25.5	M	81
S	7.0	25.0	F	90
S	8.0	26.5	F	95
S	6.5	24.5	M	130
S	7.0	25.5	F	428
S	5.5	25.0	F	790
S	7.3	25.5	F	200
S	13.5	34.0	M	10
S	5.5	24.8	F	1500
S	4.0	21.0	M	1800
S	5.5	24.0	M	1250
S	7.3	27.0	F	800
S	8.3	27.5	F	110
S	7.5	26.5	M	0
S	6.5	25.3	F	1241
S	7.0	25.8	M	720
S	4.0	21.0	M	150
S	5.5	24.3	M	37
S	4.0	21.0	M	1920
S	5.5	25.0	F	1450
S	4.5	22.5	M	300
S	7.0	25.0	M	275
S	7.5	26.0	M	500
S	6.5	24.5	M	490
Pink	4.8	21.8	M	0
P	4.8	22.3	F	0
S	4.5	22.3	M	370
S	6.8	25.3	F	580
S	6.5	24.3	M	1300
S	6.5	26.0	F	1250
S	3.5	20.5	F	750
S	8.3	26.8	F	81
S	13.9	30.3	F	0

Appendix Table 1. Continued

Species	Weight in pounds	Fork length in inches	Sex	Number of cysts per kidney
S	9.0	27.3	M	15
S	6.5	26.0	M	590
C	10.3	27.3	M	70
S	6.8	25.5	F	320
S	5.0	23.5	M	1370
S	7.5	26.0	M	1210
S	8.5	27.5	F	0
S	8.5	27.3	F	0

Table Measurements in microns of metacercarial cysts from experimentally infected fish.[1]

Host species	Dia. of cyst Max.	Dia. of cyst Min.	Thickness of cyst wall	Length of stylet	Excr. bladder Max.	Excr. bladder Min.	Approx. age of cyst in days
Steelhead	135	112	1.0	13.2	--[2]	--[2]	1
	109	106	1.2	14.9	--	--	1
	129	116	1.0	13.5	--	--	1
	122	120	1.0	13.5	--	--	1
	124	115	1.0	13.8	--	--	1
Cottid	132	130	1.0	9.0	--	--	3
	128	125	1.0	9.8	--	--	3
	111	110	1.0	8.0	--	--	3
	141	140	1.5	7.8	76	66	3
	119	116	1.0	9.0	--	--	3
Atlantic salmon	139	139	0.9	6.0[3]	--	--	5
	149	125	1.5	---	--	--	5
	139	135	1.0	---	--	--	5
	142	139	1.0	---	--	--	5
	139	116	1.0	---	--	--	5
Lake trout	132	132	0.9	6.0	--	--	5
	149	142	1.0	4.0	115	59	5
	135	116	1.5	6.0	--	--	5
	139	132	1.8	---	66	66	5
	189	175	1.0	4.0	115	59	5
Rainbow trout	135	122	1.0	7.0	--	--	5
	149	129	1.5	---	109	76	5
	142	142	1.5	8.0	102	79	5
	145	139	1.5	9.0	116	63	5
	139	125	1.0	9.0	--	--	5
	158	145	1.5	7.0	114	83	7

Table 8. Continued

Host species	Dia. of cyst Max.	Dia. of cyst Min.	Thickness of cyst wall	Length of stylet	Excr. bladder Max.	Excr. bladder Min.	Approx. age of cyst in days
Rainbow trout	145	143	2.0	7.0	92	63	7
	149	130	2.0	6.6	118	59	7
	148	144	2.5	7.0	89	63	7
	154	152	2.0	8.0	99	95	7
	139	125	1.0	5.5	83	46	5
Cottid	149	132	2.0	7.0	66	40	11
	139	130	1.5	---	--	--	11
	145	132	2.0	6.0	66	50	11
	149	139	2.0	6.0	--	--	11
	139	132	1.5	7.0	--	--	11
Gambusia	238	218	2.0	5.0	116	79	11
	182	172	2.0	---	129	59	11
	195	182	2.0	6.0	116	83	11
	215	195	2.0	5.0	122	99	11
	228	195	2.0	5.0	132	95	11
Lake trout	151	147	3.0	6.0	122	60	11
	129	123	3.0	6.0	116	63	11
	150	143	3.5	5.5	122	76	11
	136	134	3.5	5.0	99	49	11
	156	151	3.5	5.5	105	76	11
Rainbow trout	158	145	3.0	6.8	102	46	15
	211	208	3.0	7.0	149	69	15
	211	201	3.0	5.1	142	66	15
	189	173	3.3	5.0	122	59	15
	165	158	3.3	5.5	83	76	15

Table 8. Continued

Host species	Dia. of cyst Max.	Dia. of cyst Min.	Thickness of cyst wall	Length of stylet	Excr. bladder Max.	Excr. bladder Min.	Approx. age of cyst in days
Atlantic salmon	189	173	3.0	5.0	116	66	15
	158	155	2.5	5.0	112	59	15
	173	165	2.8	5.2	116	66	15
	173	168	2.8	6.6	122	59	15
	188	182	2.8	4.5	132	82	15
Rainbow trout	149	139	3.0	---	92	59	20
	162	158	3.3	3.3	109	82	20
	158	155	3.3	4.0	116	66	20
	165	158	5.0	3.5	132	109	20
	162	162	3.0	---	89	59	20
Atlantic salmon	173	165	5.0	---	99	89	20
	168	165	5.0	5.0	109	79	20
	162	152	4.0	3.3	83	33	20
	164	157	4.0	3.5	100	72	20
	170	165	5.0	4.0	99	88	20
	198	188	6.0	---	116	106	25
	215	211	6.0	---	132	50	25
	205	201	5.0	3.3	122	69	25
	201	198	4.0	---	139	83	25
	198	188	5.0	---	132	66	25
	208	206	6.0	---	139	83	30
	201	198	6.0	---	135	66	30
	188	188	5.0	---	112	59	30
	211	205	6.5	---	122	73	30
	201	198	5.0	---	116	74	30

Table 8. Continued

Host species	Dia. of cyst Max.	Dia. of cyst Min.	Thickness of cyst wall	Length of stylet	Excr. bladder Max.	Excr. bladder Min.	Approx. age of cyst in days
Lake trout	219	217	6.5	---	132	85	30
	220	220	6.5	---	148	93	30
	204	201	5.5	---	129	70	30
	209	205	5.5	---	131	63	30
	205	202	5.0	---	128	76	30
	233	230	6.6	---	137	116	45
	237	233	6.0	5.0	132	92	45
	230	230	6.0	---	139	99	45
	251	248	5.0	3.0	139	116	45
	237	233	6.0	---	132	99	45
Atlantic salmon	221	212	7.5	---	139	92	50
	219	216	7.5	---	147	79	50
	219	208	7.0	---	144	69	50
	241	237	7.0	---	142	93	50
	252	249	7.5	---	133	100	50
	232	229	8.0	---	142	99	106
	248	240	8.0	---	149	106	106
	231	227	8.0	---	139	83	106
	237	235	8.0	---	150	102	106
	248	242	7.5	---	140	99	106

[1] The fish were infected by exposing them to infected snails for three to five days

[2] Excretory bladder not filled

[3] Stylet absent