

AN ABSTRACT OF THE THESIS OF

DUANE WILLIAM KARNA for the MASTER OF SCIENCE  
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Title: EPIZOOTIOLOGY OF MARGARITIFERA MARGARITIFERA  
(L.) (MOLLUSCA: MARGARITANIDAE) INFECTION IN  
SALMONID FISHES

Abstract approved: **Redacted for privacy**  
Dr. Raymond E. Millemann

Glochidial development in freshwater mussels (Margaritifera margaritifera) located in the Siletz River, Oregon, was completed in 13 days at an average water temperature of 12.8 C. Glochidia were released by these mussels for 33 days, May 13 to June 15, 1971.

The comparative susceptibility of four species of salmonid fishes, 30.5 to 87.0 mm in fork length, to glochidiosis was determined by examination of 343 caged and 177 free-swimming (native) fish for infection. Of the caged fish, 99% of the chinook salmon (Oncorhynchus tshawytscha), 75% of the coho salmon (O. kisutch), 88% of the cutthroat trout (Salmo clarki), and 95% of the steelhead trout (S. gairdneri) were infected. There was a similar relationship in infection incidence in the native fish species. Mean infection intensities in the caged and native fish were: 446 and 399 for chinook

salmon, 8 and 24 for coho salmon, and 72 and 88 for steelhead trout, respectively, and 212 for caged cutthroat trout (native trout were not captured).

This is the first detailed description of the metamorphosis of M. margaritifera glochidia in fish and the associated histopathology. Invading glochidia, which were 70 by 75  $\mu$  in size, increased in length by 500% during metamorphosis. Encysted glochidia occurred on the gill filaments, arches, rakers, and occasionally on the pseudo-branches of all fish species; however, most were on the lamellae of the filaments. Initially, the encysted glochidia have uneven walls approximately 15  $\mu$  in thickness, but as the parasite increases in size the outer wall, i. e., the part of the cyst that is not embedded in and surrounded by gill lamellae, becomes thinner. Also, approximately 15 gill lamellae may become fused to the wall. Except for the lamella grasped by the glochidium, blood apparently continues to flow through the capillaries of the fused lamellae. However, these lamellae apparently can no longer function in respiration, except for the outermost lamella. Parasites encysted on the side of the gill filament restrict blood flow by pinching the filamental arteries. Large cysts on the lamellae increased the physiological dead space in the water flow. Clubbing of the filaments results when large cysts are located near the distal end of the filament. These pathological changes in heavy infections may result in immediate death of the fish

by asphyxiation. In less heavy infections, delayed mortality occurs due to secondary infection with fungi, probably Saprolegnia sp. The invading or exiting glochidia may provide portals of entry for the fungi.

Epizootiology of Margaritifera margaritifera (L.)  
(Mollusca: Margaritanidae) Infection in Salmonid Fishes

by

Duane William Karna

A THESIS

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I appreciate the patience and support of my wife, Karen, and I apologize to my two children, Heidi and Kristen, for being absent from our home on many evenings and weekends during this study.

## TABLE OF CONTENTS

|  | <u>Page</u> |
|--|-------------|
| INTRODUCTION   | 1           |
| Distribution   | 1           |
| Ecology  | 2           |
| Life History   | 3           |
| Host-Parasite Relationship                                       | 6           |
| Natural Infections   | 6           |
| Experimental Infections  | 7           |
| Objectives   | 8           |
| <br>MATERIALS AND METHODS  | <br>10      |
| Description of the Study Area                                    | 10          |
| Field Apparatus  | 11          |
| Field and Laboratory Procedures                                  | 12          |
| <br>RESULTS  | <br>18      |
| Glochidial Development   | 18          |
| Susceptibility of Fish to Glochidiosis                           | 23          |
| Effects of Glochidiosis on Captive Fish                          | 31          |
| Relationship Between Condition Factor and<br>Infection Intensity | 31          |
| Mortalities  | 32          |
| Metamorphosis and Histopathology                                 | 40          |
| <br>DISCUSSION   | <br>55      |
| <br>BIBLIOGRAPHY   | <br>63      |

## LIST OF FIGURES

| <u>Figure</u> |   | <u>Page</u> |
|---------------|---|-------------|
| 1             | Diagram of one of the four suspended cages that were used to hold the captive fish.   | 13          |
| 2             | Average daily water temperature recorded near river mile 21 in the Siletz River, Oregon, 1971.  | 21          |
| 3             | Mean infection levels in chinook salmon and cutthroat trout exposed continuously to the glochidia of <u>Margaritifera margaritifera</u> .     | 24          |
| 4             | Mean infection levels in coho salmon and steelhead trout exposed continuously to the glochidia of <u>Margaritifera margaritifera</u> .        | 25          |
| 5             | Cumulative infection levels in four salmonid species exposed continuously to the glochidia of <u>Margaritifera margaritifera</u> .            | 29          |
| 6             | Relationship between intensity of glochidial infection and condition factors for chinook salmon.  | 33          |
| 7             | Relationship between intensity of glochidial infection and condition factors for cutthroat trout.   | 34          |
| 8             | Relationship between intensity of glochidial infection and condition factors for steelhead trout.   | 35          |
| 9             | Daily mortalities of four salmonid species exposed continuously to the glochidia of <u>Margaritifera margaritifera</u> .                      | 38          |
| 10, 11        | Photomicrographs of cross sections of recently encysted <u>Margaritifera margaritifera</u> glochidia in the gill filaments of chinook salmon. | 42          |
| 12            | Photomicrograph of a cross section of a <u>Margaritifera margaritifera</u> glochidium encysted in the gill filament of a chinook salmon.      | 44          |

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## LIST OF TABLES

| <u>Table</u> |  | <u>Page</u> |
|--------------|--|-------------|
| 1            | Relationship between glochidial development and water temperature in mussels collected in the Siletz River, Oregon, from February 20 to June 17, 1971.   | 19          |
| 2            | Incidence and intensity of glochidial infection in live captive fish.  | 26          |
| 3            | Incidence and intensity of glochidial infection in native chinook salmon, coho salmon, cutthroat trout, and steelhead trout collected near river mile 21 in the Siletz River from May 22 to June 19, 1971. | 27          |
| 4            | Incidence and intensity of glochidial and fungal infections in dead captive fish.  | 37          |

EPIZOOTIOLOGY OF MARGARITIFERA MARGARITIFERA (L.)  
(MOLLUSCA: MARGARITANIDAE) INFECTION  
IN SALMONID FISHES

INTRODUCTION

Distribution

The distribution of the freshwater mussel Margaritifera margaritifera (L.), which is holarctic, is more extensive than that of any other freshwater mussel (Jackson, 1925; Ingram, 1948). This mussel occurs in Portugal, Spain, France, Belgium, Germany, British Isles, Denmark, Norway, Sweden, Finland, and Russia in Europe, and also in Iceland, and in eastern Asia it inhabits Eastern Siberia, the Amur River region, Mongolia, Kamchatka, Sakhalin Island, and Japan. In North America, this species is found from Newfoundland to about latitude 40° N on the east coast, and from Naha Bay, Alaska to about latitude 35° N on the west coast (Dall, 1905; Walker, 1910; Jackson, 1925; Roscoe and Redelings, 1964). Ingram (1948) determined the general range of M. margaritifera in Washington, Oregon, and California, but the distribution he gives for Oregon is incomplete because it omits the Siletz, Yaquina, Elk, Alsea, and Millicoma rivers on the coast, and the Long Tom, Luckiamute, Deschutes, and Riddle rivers inland. Specimens of M. margaritifera collected from these rivers are in the Invertebrate

Collection of the Department of Fisheries and Wildlife at Oregon State University.

### Ecology

The ecology of M. margaritifera is not well known. Murphy (1942) reported that the stable sand-gravel stretches of the Truckee River near Truckee, California, were ideal habitat for this mussel. He estimated one bed to contain 10,000 individuals. Hendelberg (1960) reported that in central and northern Europe this mussel is usually found in cool, swift flowing waters, which are low in dissolved calcium carbonate, and in fast currents they may be found behind large boulders. He also found that 90% of the mussels he collected in Northern Sweden occurred in water depths of 0.5 to 1.5 m. Roscoe and Redelings (1964) reviewed the literature and also made some preliminary observations of a bed of several thousand mussels in the Kettle River near Barstow, Washington. They found that the bed was confined to an area of swift water flow; the water depth ranged from 0.6 to 1.2 m at the lowest level, and the substrate consisted mainly of boulders, gravel, and sand. Stober (1972) studied the distribution of M. margaritifera in a bed in the Madison River in western Montana. He found that this mussel was usually restricted to scoured depressions having substrates ranging from coarse sand to 10- to 15-cm rubble. The water velocity in these depressions ranged

from 0.12 to 0.21 meters per second at 0.02 m above the substrate, and all mussels were collected in water that ranged in depth from 0.5 to 0.8 m.

In Japan, Okada and Koba (1933) observed M. margaritifera buried to two-thirds their length and angled 40 to 80° into the current. These animals generally remain stationary under favorable water current conditions (Murphy, 1942); however, they can move 4.5 m per day (Jackson, 1925). Altnoder (1926) reported an upstream movement of 22 m in 2 years.

#### Life History

In late May or early June, the eggs of M. margaritifera pass from the ovary into the four brood chambers (marsupia), each located within one of the gills (Murphy, 1942). The marsupia of a mussel, which was 70 mm long, contained approximately 1 million eggs (Murphy, 1942). Sperm are liberated into the water from the male's excurrent siphon and are carried by the current to the female where they may be drawn into her incurrent siphon. They then enter the marsupia and fertilize the eggs. The marsupia eventually become distended as a result of larval development (Pennak, 1953). Jackson (1925) and Riedl (1928) stated that development from unfertilized eggs to mature larvae (glochidia) took from 16 to 28 days depending on the water temperature. Murphy (1942) reported a maturation time of

approximately 12 days at a water temperature of 13.1 C. Choi and Choi (1965) found that larval development of a related mussel (Lamprotula coreana) was also temperature dependent. They observed glochidial formation after 15, 12, 7, and 5 days at 10, 15, 20, and 25 C, respectively.

Murphy (1942) reported that glochidia of M. margaritifera, which measured 50 to 60  $\mu$  in length, leave the marsupia and are free-living for periods up to 11 days, but he did not state the criteria he used to determine their viability. Tedla and Fernando (1969) found that the glochidia of a related mussel (Lampsilis radiata siliquoidea) survived for 9 days at 10 C, but at 20 C none survived beyond 24 hr. They used infectivity of the glochidia for fish as the criterion of viability.

Sometime during their free-living period, the glochidia of most freshwater mussels must attach to the gills, skin, or fins of a suitable fish host to undergo metamorphosis to the adult stage (Pennak, 1953). Unlike some of the other species of freshwater mussels, the glochidia of M. margaritifera are hookless; however, they do have six or seven minute teeth on the ventral rim of their calcareous shell that presumably aid in attachment (Jackson, 1925). Harms (1909) found that these glochidia were exclusively gill parasites. Reidl (1928) stated that the glochidia of M. margaritifera became encysted in a thick cyst in the gills of fish in 2 to 4 hr. Arey (1932a) studied

the encystment of the hookless glochidia of Lampsilis luteola on the gills of the largemouth bass (Micropterus salmoides). He found that they were covered by migrating epithelial and stromal cells in 2.5 to 3.5 hr depending on water temperature. Similarly, D'Eliscu (1972) found that at 20 C the glochidia of Anodonta californiensis became encysted in 3 to 4.5 hr on the gills and fins of mosquitofish (Gambusia affinis). Murphy (1942) reported that the glochidia of M. margaritifera remained encysted in laboratory-infected rainbow trout (Salmo gairdneri) and brown trout (S. trutta) for 36 days at an average water temperature of 14 C. Tedla and Fernando (1969) found that at 15 C the parasitic stage of L. radiata siliquoidea on the gills of experimentally-infected yellow perch (Perca flavescens) lasted 40 to 50 days. At the end of metamorphosis of M. margaritifera, Murphy (1942) found that the glochidia increased in length by approximately 660% at the expense of host tissue. Arey (1932b) observed the breakdown of larval structures and of gill tissue pinched between the larval shells during the metamorphosis of L. luteola; however, in this species there is no growth of the mussel during its parasitic stage.

After metamorphosis, the juvenile mussel of M. margaritifera leaves the fish by using its foot to rupture the cyst wall (Murphy, 1942). They, like many other freshwater mussels, then settle on the stream bottom and attach to the substrate by byssal threads

(Pennak, 1953). The byssus of M. margaritifera is lost when the mussel reaches approximately 50 mm in length (Murphy, 1942).

M. margaritifera becomes sexually mature at approximately 30 mm in length (Murphy, 1942). The sexes are usually separate and indistinguishable externally (Hendelberg, 1960). Wellmann (1938) found one hermaphrodite among 80 mussels he examined in Germany, and Van der Schalie (1970) found one hermaphrodite among 12 specimens collected from Yellowstone National Park, Wyoming.

Comfort (1957) and Hendelberg (1960) stated that M. margaritifera is one of the longest-lived of all invertebrates. They estimated that adults may live for more than 100 years. Comfort's age estimates were based on the extremely slow growth rate, and Hendelberg counted annual layers in sagittal sections of hinge ligaments. Using Hendelberg's technique, Stober (1972) found that the average and maximum ages of 84 mussels were 47.9 and 67 years, respectively.

### Host-Parasite Relationship

#### Natural Infections

The following fish species have been found naturally infected with the glochidia of M. margaritifera: brown trout in Europe (Wilson, 1916), rainbow and brown trout in California (Murphy,

1942), and chinook salmon (Oncorhynchus tshawytscha) in Washington (Davis, 1953). Also, Harms (1907) reported that several kinds of minnows (species unknown) in Europe were naturally infected with these glochidia. There is no further information on the natural fish hosts for M. margaritifera.

There is no information on the effects on fish of natural infections with any species of glochidia. Tedla and Fernando (1969) examined 1,620 yellow perch infected with L. radiata but reported only the incidence and intensity of infection.

#### Experimental Infections

Murphy (1942) experimentally infected rainbow trout, brown trout, brook trout (Salvelinus fontinalis), Tahoe suckers (Catostomus tahoensis), "black minnows" (Rhinichthys osculus robustus), and the Lahontan redbreast (Richardsonius egregius) with the glochidia of M. margaritifera. Mountain whitefish (Prosopium williamsoni) and the Piute sculpin (Cottus beldingi) were resistant to infection. All fish were less than 7.0 cm long.

Murphy (1942) studied the effects of glochidiosis on experimentally-infected rainbow trout, averaging 38 and 42 mm in length, and brown trout, averaging 31 mm in length. Of the 400 rainbow trout infected with 600 to 1,200 glochidia, 52% died within 1 day after exposure. Death presumably was due to interference of gill

circulation by the glochidia. Mortalities of rainbow trout infected with 250 to 750 glochidia and brown trout infected with 100 to 295 glochidia occurred approximately 40 days after exposure. Cumulative mortalities at day 40 for rainbow trout and brown trout were 16 and 52%, respectively. Deaths were attributed to emaciation or secondary bacterial or fungal infections after the parasites left the fish. There were no deaths of rainbow trout infected with 30 to 50 glochidia. The only other recent study on effects of experimental glochidial infections on fish is that of Tedla and Fernando (1969). They reported no deaths of yellow perch exposed to several thousand glochidia of L. radiata siliquoidea. Lefevre and Curtis (1912) experimentally infected largemouth bass with the hookless glochidia of the freshwater mussel Lampsilis ligamentina. With infection levels estimated at 2,000 to 2,500 glochidia in fish 10.2 to 12.7 cm in length, 55% of the fish died in 30 days. Cause of death was attributed to hypertrophy of the gill tissue. In another experiment, Lefevre and Curtis (1912) observed slight mortality in 10.2-cm rock bass (Ambloplites rupestris) infected with an estimated 2,500 L. ligamentina glochidia.

### Objectives

The general objective of my thesis was to study, under field conditions, certain aspects of the host-parasite relationship between

glochidia of M. margaritifera and four species of alevin salmonid fishes. Specific objectives were: (1) to determine if there is a relationship between glochidial development in adult mussels and water temperature; (2) to determine the comparative susceptibility of chinook salmon, coho salmon (O. kisutch), cutthroat trout (S. clarki), and steelhead trout held captive in a stream and in native (free-swimming) fish from the same stream to natural glochidial infections; (3) to determine the effects of the infection on the captive fish; and (4) to describe the morphological changes that occur during metamorphosis of the glochidia.

## MATERIALS AND METHODS

Alevin salmonid fish were used for the field study. Fall chinook salmon and coho salmon were obtained from the Bonneville and Alsea River Salmon Hatcheries, respectively, of the Fish Commission of Oregon. Cutthroat trout and winter steelhead trout were obtained from the Oregon State Game Commission's Alsea Trout Hatchery. The mean fork lengths with the ranges in parenthesis for each species were: chinook salmon, 64.0 mm (52.5 to 77.0 mm); coho salmon, 59.0 mm (41.5 to 87.0 mm); cutthroat trout, 55.5 mm (41.5 to 73.0 mm); steelhead trout, 51.0 mm (38.5 to 57.5 mm).

### Description of the Study Area

The study area was near river mile 21 on the Siletz River in western Oregon. It was 365 m long and averaged 35 m in width and extended from a small island to a deep pool downstream. The stream bottom was nearly flat and consisted of a stable sand-gravel substrate. Water depth varied from approximately 1.0 m in the summer to at least 4.5 m during the winter, and fluctuated 0.3 m or more with the tidal cycle.

An estimated 100,000 M. margaritifera adults inhabit this section of the river. They are concentrated on the bottom in rows perpendicular to the stream flow. Each row is approximately 1.0 m

wide and many extend almost the entire width of the river. Densities exceeding 600 adult mussels per square meter were counted, and densities estimated to exceed 400 mussels per square meter were common. Other invertebrates observed in the study area were snails (Oxytrema silicula) and crayfish (Pacifastacus trowbridgi).

Native fish found in or near the area during the time of the study were alevin chinook salmon, alevin coho salmon, alevin and adult steelhead trout, adult cutthroat trout, prickly sculpins (C. asper), threespine sticklebacks (Gasterosteus aculeatus), largemouth bass, Pacific lampreys (Entosphenus tridentatus), and the western brook lamprey (Lampetra richardsoni).

#### Field Apparatus

Water temperature was recorded continuously from March 6 to July 24, 1971, on a Ryan thermograph located on the stream bottom in the center of the study area. The thermograph was housed in a perforated pipe 50.8 cm long and 10.2 cm in diameter. One end of the pipe was sealed with a steel plate, and the other end was fitted with a lockable cover. A cable secured the thermograph housing to a tree located on the bank.

Four 76-cm-square cages with wooden frames and covered with 3.2-mm (1/8-inch) hardware cloth were constructed to hold the captive fish. They were placed at 4.5 m intervals in the deep pool

on the downstream edge of the study area. Each cage was suspended approximately 1.5 m below the water surface on two 3.2-mm (1/8-inch) steel cables (Fig. 1). These cables were strung between an overhead 6.4-mm (1/4-inch) steel cable and a 50-kg concrete anchor. A cable clamp attached to the two vertical cables locked each cage in position.

### Field and Laboratory Procedures

Adult mussels were collected from the study area biweekly from February 20 to April 17, weekly from April 24 to May 13, and about every 4 days from May 16 to June 17, 1971. Except for the last two collections, which consisted of 524 and 625 mussels, the sample size ranged from 12 to 56 and averaged about 30 mussels. A total of 317 mussels were examined in the laboratory for determination of sex by microscopic examination of wet mounts of gonadal tissue, and for glochidial development by similar examination of the marsupial tissue. In the field, 1,319 mussels were examined for distended marsupia to determine if they were gravid. I will use the term gravid to mean the presence of eggs, early larval stages, or glochidia in the marsupia of female mussels.

On May 15, 1971, groups of 325 chinook salmon, coho salmon, cutthroat trout, and steelhead trout were placed in the suspended cages, one group per cage. A sample of seven live fish was taken

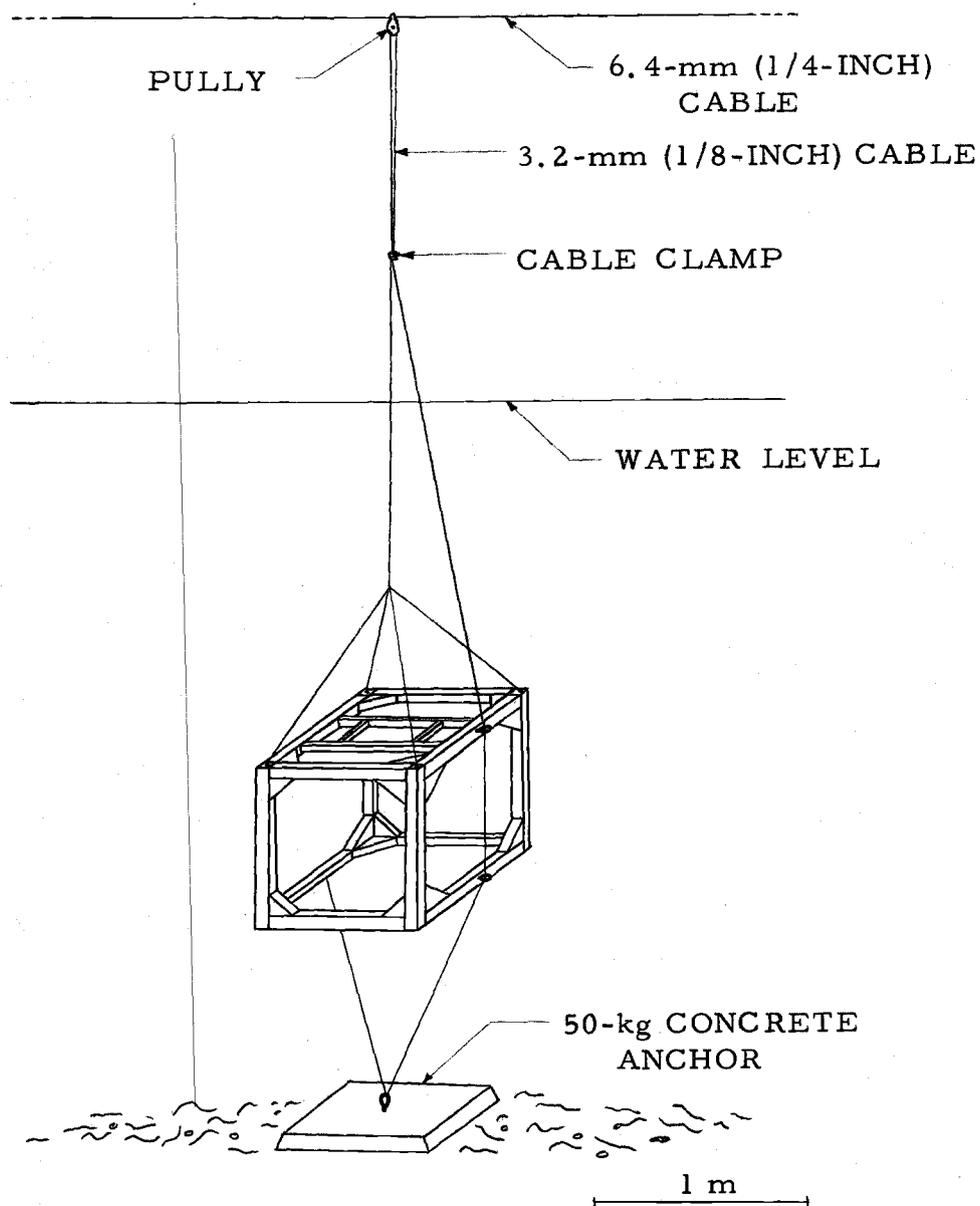


Figure 1. Diagram of one of the four suspended cages that were used to hold the captive fish.

from each cage every 2 days from May 16 through June 11, every 4 days from June 11 through June 23, and thereafter, every 8 days until no fish remained or until July 17 when the experiment was terminated. The fish were transported to the laboratory and kept alive in aquaria or immediately preserved in 10% buffered neutral Formalin. Two live fish from each sample were placed in Bouin's fixative for subsequent histological examination. The glochidia in the gills of the remaining five fish were counted either immediately after killing the fish or after they had been preserved in 10% neutral Formalin. Dead fish were also removed from the cages at the time of sampling and were immediately preserved in 10% neutral Formalin.

Fish native to the Siletz River were captured on or near the study area by handnet, seine, or scoop trap. They were preserved in 10% neutral Formalin until examined subsequently for glochidial infection.

Gills were dissected from the fish and placed in water in Petri dishes. The glochidia in each holobranch were counted under a dissecting microscope. Glochidia were not counted in fish that had bilateral fungal infections of the holobranchs. If the infection was unilateral, then the glochidia in the uninfected gills were counted, and the count was doubled to obtain an estimate of the total number of glochidia present. The glochidia in most of the dead fish and in 12 of the live captive fish were counted in this manner. These estimated

totals will be indicated in the text by the notation 2X in parenthesis after the number of glochidia.

Condition factors (CF) for the live captive fish were calculated using a modification of Bennett's (1962) formula:

$$CF = \frac{W(10^5)}{L^3}$$

where weight (W) is to the nearest 0.1 g and fork length (L) to the nearest 0.5 mm. Some live fish taken from the cages were preserved in Formalin prior to measurement. Since preserved fish shrink in length and gain weight (Parker, 1963), correction factors were calculated from weights and lengths of fish taken before and after their preservation in Formalin for 30, 60, and 90 days. Two groups of 10 and 15 fish were used in this test. The correction factors in the following formula are based on an average preservation time of 54 days:

$$CF = \frac{W - 0.13W(10^5)}{(L + 0.04L)^3} .$$

Condition factors were compared with infection intensities in captive chinook salmon, cutthroat trout, and steelhead trout for exposure days 5 through 31. Infection intensities in the coho salmon were too low to make such a comparison, e. g., 89% of these fish had less than 15 encysted glochidia. For the same reason, condition

factors were not computed for the other fish species collected on exposure days 1 and 3. Condition factors were not computed for fish collected after day 31 because glochidia were no longer seen in the marsupia of female mussels. Condition factors were not calculated for the dead fish because of their decomposition. The stomachs and intestines were removed from these fish and examined for the presence of food to determine if death may have been due to starvation.

The metamorphosis and associated histopathology of glochidial infections was studied from gills of captive fish. Gill tissue was taken from each fish species on the following days of exposure: chinook salmon-days 1, 5, 15, 23, 31, 35, and 39; coho salmon-days 9, 35, and 39; cutthroat trout-days 13, 15, 35, and 47; and steelhead trout-days 5, 15, 35, and 39.

Holobranchs were removed from these fish and examined under the dissecting microscope to check for the presence of glochidia. Normal gill tissue was obtained from uninfected laboratory-reared fish of each species. Serial sections 7 or 8  $\mu$  thick were made in paraffin and stained in hemotoxylin and eosin. All tissues were sectioned longitudinally and some also transversely. Gill arches and filaments were present in both sections.

I could not determine the age of encysted glochidia in the naturally-infected fish because the exact time of infection was

unknown. Therefore, I chose arbitrarily four stages of development to describe the metamorphosis and associated histopathology.

Recently encysted glochidia constituted the first stage; larger parasites that measured 120 and 210  $\mu$  in cross-sectional height were the second and third stages, respectively; and the fourth stage was defined as parasites that measured 240  $\mu$  or more in length. Cross-sectional measurements were made from the hinge ligament to the ventral edge of the larval shell, and length was measured at the greatest distance perpendicular to the height measurement in lateral view. All measurements were made using a compound microscope with an ocular micrometer.

## RESULTS

Glochidial Development

In most samples, the sex distribution of adult mussels was approximately equal (Table 1). Mussels collected after May 24, 1971, were examined in the field, and only the number of gravid females, which were identified by their distended marsupia, was recorded. Eggs were not present in the marsupia of mussels collected before April 30 (Table 1). On April 30, eggs were found in the marsupia of 2 of 20 mussels. From May 7 to May 24, 66 to 100% of the female mussels were gravid; thereafter, the number of gravid mussels declined gradually until June 17 when no gravid females were found in a sample of 625 mussels of unknown sex (Table 1). In the May 7 collection, the sex of 18 of 20 mussels could not be determined, but many of them undoubtedly were gravid because of the large numbers of aborted gametes and immature larvae present in the water used to transport these animals to the laboratory. Also, some of these mussels may have already released their sperm or glochidia naturally. To reduce abortion in subsequent samples, the mussels were layered between cloth towels and ice cubes in an ice chest for transportation to the laboratory.

Glochidia were first observed in 2 of 11 females collected on May 13, and the marsupia of the other females contained either eggs

Table 1. Relationship between glochidial development and water temperature in mussels collected in the Siletz River, Oregon, from February 20 to June 17, 1971.

| Collection Date | Sample Size      | Sex <sup>a</sup> |     |   |    | No. of Gravid Females <sup>b</sup> | Mean Water Temperature (C) |
|-----------------|------------------|------------------|-----|---|----|------------------------------------|----------------------------|
|                 |                  | M                | F   | H | U  |                                    |                            |
| Feb. 20         | 12               | 5                | 7   | 0 | 0  | 0                                  | 6.7                        |
| Mar. 6          | 34               | 21               | 13  | 0 | 0  | 0                                  | 6.1                        |
| 20              | 30               | 13               | 16  | 1 | 0  | 0                                  | 7.8                        |
| Apr. 3          | 15               | 8                | 7   | 0 | 0  | 0                                  | 8.5                        |
| 17              | 37               | 26               | 11  | 0 | 0  | 0                                  | 8.5                        |
| 24              | 27               | 10               | 17  | 0 | 0  | 0                                  | 9.3                        |
| 30              | 56               | 36               | 20  | 0 | 0  | 2                                  | 10.5                       |
| May 7           | 20               | 1                | 1   | 0 | 18 | 1                                  | 12.0                       |
| 13              | 17               | 6                | 11  | 0 | 0  | 11                                 | 14.5                       |
| 16              | 29               | 13               | 15  | 0 | 1  | 10                                 | 12.0                       |
| 20              | 20               | 5                | 9   | 0 | 6  | 9                                  | 11.5                       |
| 24              | 20               | 7                | 7   | 0 | 6  | 7                                  | 15.3                       |
| 28              | 20 <sup>c</sup>  | -                | -   | - | -  | 5                                  | 14.4                       |
| Jun. 1          | 50 <sup>c</sup>  | -                | -   | - | -  | 6                                  | 12.5                       |
| 5               | 50 <sup>c</sup>  | -                | -   | - | -  | 2                                  | 14.0                       |
| 9               | 50 <sup>c</sup>  | -                | -   | - | -  | 2                                  | 15.2                       |
| 15              | 524 <sup>c</sup> | -                | -   | - | -  | 2                                  | 15.0                       |
| 17              | 625 <sup>c</sup> | -                | -   | - | -  | 0                                  | 15.9                       |
| Totals          | 1,636            | 151              | 134 | 1 | 31 | 57                                 |                            |

(a) M = male, F = female, H = hermaphrodite, U = unknown. Sex was determined by microscopic examination of wet mounts of gonadal or marsupial tissues.

(b) Females with eggs, immature larvae, or glochidia in their marsupia.

(c) Examined in the field and only the number of gravid females was recorded.

or immature larvae. In subsequent collections examined in the laboratory, glochidia were present in 20 to 28% of the gravid mussels.

Water temperature averaged 8.5 C for the 54 days prior to April 30 when eggs were first observed in the marsupia. Four days before April 30 it exceeded 10 C for the first time (Fig. 2). Consecutive 10-day average temperatures from March 11 to April 29 were: 7.5, 8.0, 8.7, 8.3, and 10.3 C. The average temperature from April 30 to May 13 (13 days) was 12.8 C, and from May 13 to June 15 (33 days) it was 13.6 C. Glochidia were first and last observed in marsupia on May 13 and June 15, respectively.

These results agree with those of Murphy (1942), who found that glochidia required 12 days for development at an average temperature of 13.1 C. He also reported that at an average temperature of approximately 11 C, glochidia were present in the marsupia for 37 days.

In a separate laboratory experiment, I held gravid females in three troughs with flowing, aerated water at 5, 10, and 15 C, respectively. Each trough was 25 cm wide, 25 cm deep, and 1.8 m long and contained 7 cm of sand substrate. Immature larvae were aborted more often at 5 and 15 C than they were at 10 C. These immature larvae were released from the mussels either individually or in small groups during a period of 10 to 15 minutes. Presumably these premature larval releases were caused by some physical or

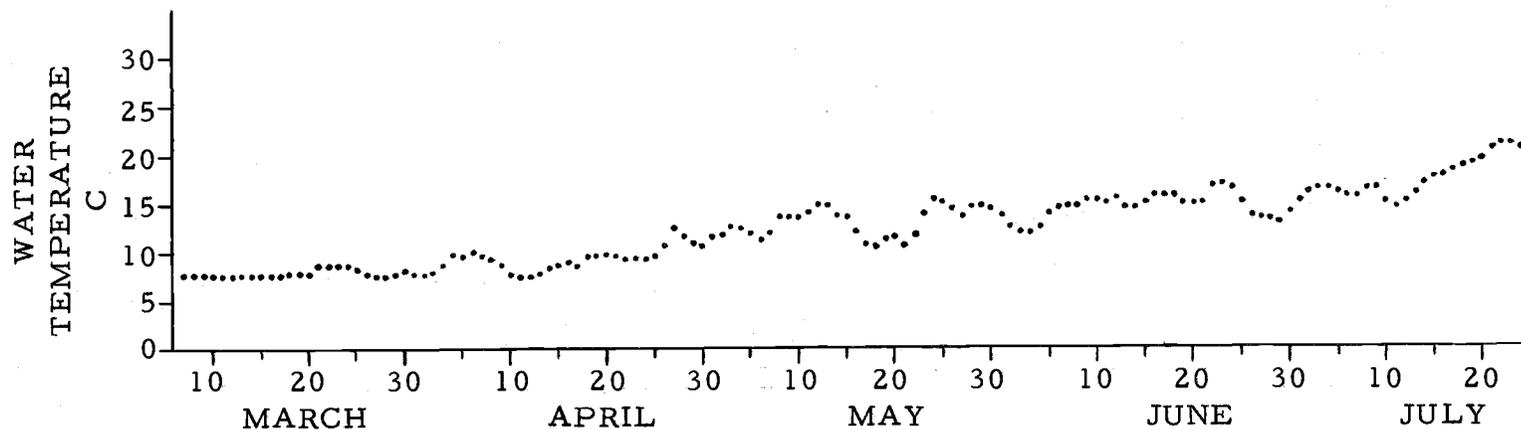


Figure 2. Average daily water temperature recorded near river mile 21 in the Siletz River, Oregon, 1971.

physiological stress, of which temperature was probably the most important. Matteson (1948) found that rough handling and sudden changes in temperature were the main causes of abortion in a related mussel (Elliptio complanatus). Murphy (1942) did not observe abortion in his study of M. margaritifera.

I observed only one natural release of glochidia. This mussel was releasing glochidia when it was removed from the river. It was immediately placed in a plastic pail containing river water, and it continued to release glochidia individually or in small groups for approximately 5 minutes. The glochidia became suspended and evenly distributed when the water was mixed slightly. Murphy (1942) observed two natural glochidial releases. In both, all glochidia were released in approximately 50 seconds as a single mass, which was then broken up by the water current.

The hookless, subovate glochidia of M. margaritifera, which are contained within a fragile egg shell when released by the female, are small compared with those of other freshwater mussels. The length and height of mature glochidia, which were obtained from gravid female mussels, ranged from 70 by 75  $\mu$  to 73 by 80  $\mu$ , respectively. The average distance across the open shell valves was 105  $\mu$ . Naturally released glochidia, which had been preserved in 10% neutral Formalin, averaged 70  $\mu$  in length by 75  $\mu$  in height. Shell height is the distance from the hinge ligament to the ventral

edge of the shell, and the length is the greatest distance perpendicular to the height.

### Susceptibility of Fish to Glochidiosis

All fish species examined, which included two nonsalmonids (prickly sculpins and threespine sticklebacks), were infected (Fig. 3, 4; Tables 2, 3). Of the captive and native fish, 509 of 594 and 174 of 194 fish were infected, respectively, with a total of 174,560 glochidia. The infection intensities in the captive fish were indicative of the relative susceptibilities of the salmonid species. Of these fish, chinook salmon was the most susceptible and coho salmon the most resistant species. On exposure day 20, chinook and coho salmon had approximate means of 900 and 10 parasites, respectively (Fig. 3, 4). Captive cutthroat trout and steelhead trout were intermediate in resistance. The maximum mean number of parasites found in these fish were 500 on exposure day 13 and 100 on exposure day 12, respectively (Fig. 3, 4).

Chinook salmon had the highest incidence and infection intensity of the captive and native salmonids (Tables 2, 3). The total and mean number of parasites present in 77 of 78 live captive fish collected during their 39-day exposure period were 34,346 and 446, respectively (Fig. 5, Table 2). The total number of parasites present in 65 of 67 live and 9 of 9 dead native chinook salmon, which

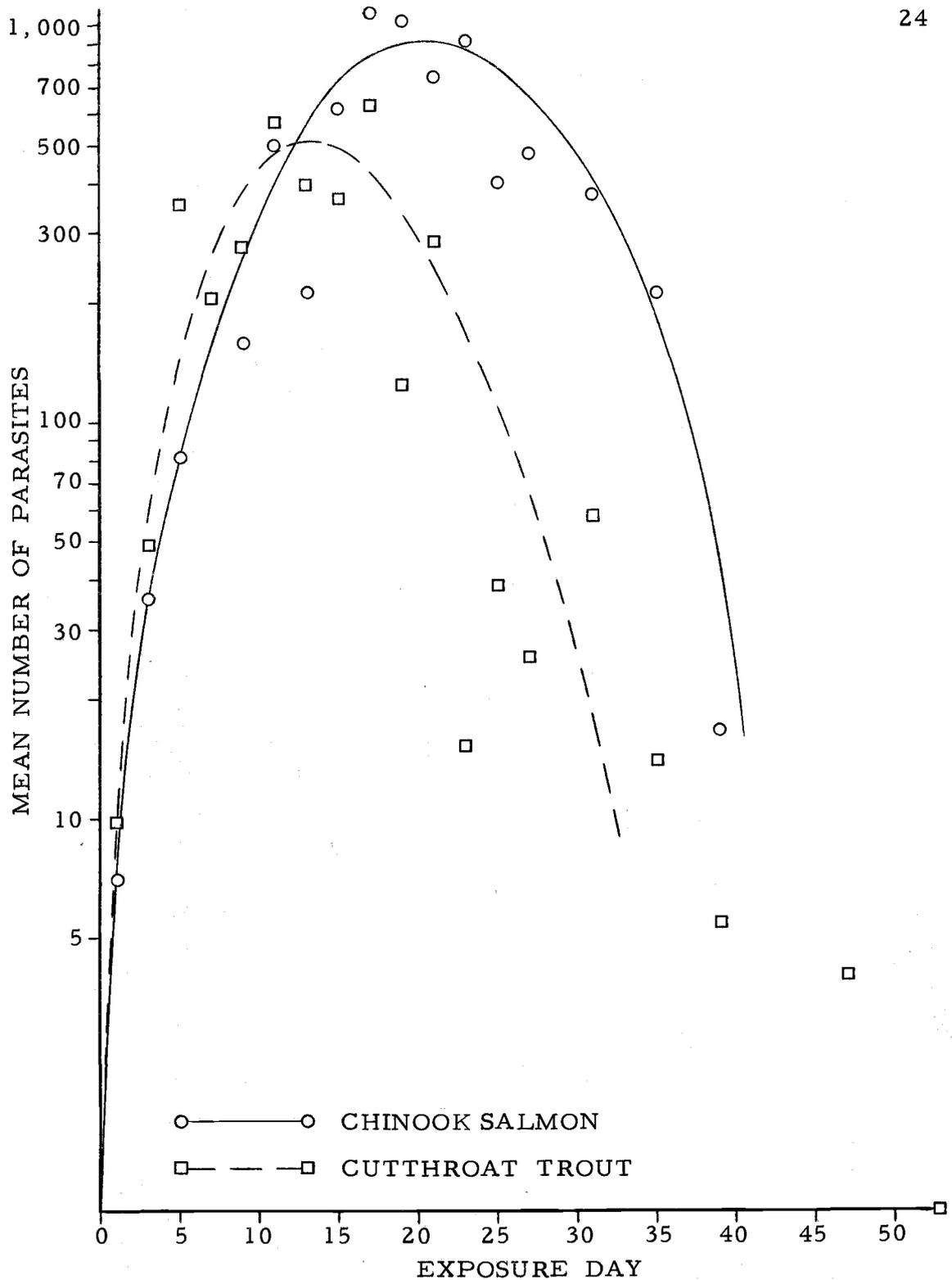


Figure 3. Mean infection levels in chinook salmon and cutthroat trout exposed continuously to the glochidia of Margaritifera margaritifera. Each point represents a sample size of five fish.

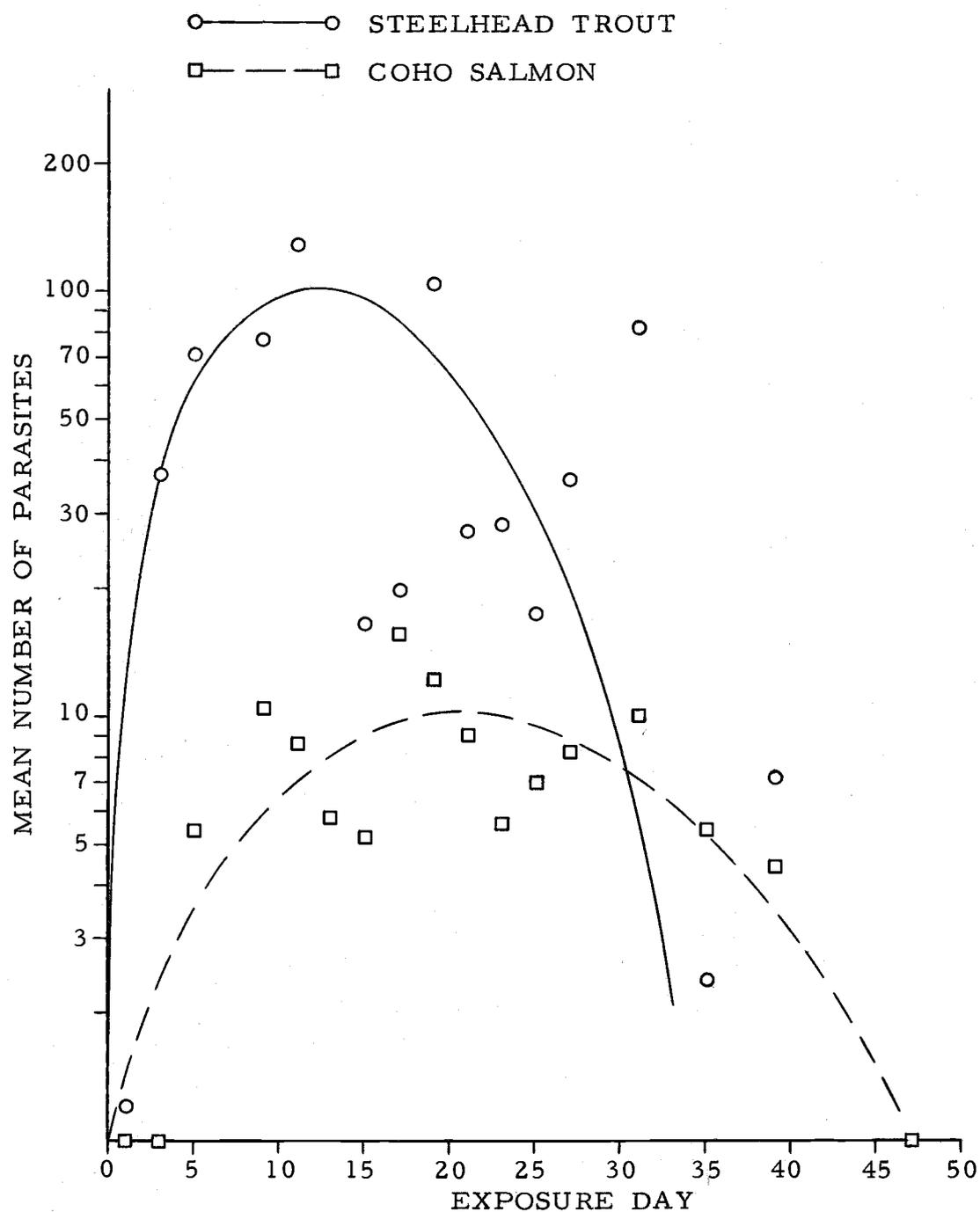


Figure 4. Mean infection levels in coho salmon and steelhead trout exposed continuously to the glochidia of Margaritifera margaritifera. Each point represents a sample size of five fish.

Table 2. Incidence and intensity of glochidial infection in live captive fish.

| Species         | Exposure period <sup>a</sup><br>(days) | Incidence <sup>b</sup> | Percent of fish with the following infection intensities: |         |         |           |         | Parasites |        |
|-----------------|--|------------------------|---|---------|---------|-----------|---------|-----------|--------|
|                 |  |                        | <100  | 100-250 | 251-500 | 501-1,000 | > 1,000 | Mean      | Total  |
| Chinook salmon  | 39                                     | 77/78                  | 36  | 13      | 19      | 17        | 15      | 446       | 34,346 |
| Coho salmon     | 63                                     | 71/95                  | 100   | 0       | 0       | 0         | 0       | 8         | 576    |
| Cutthroat trout | 55                                     | 79/90                  | 65  | 14      | 9       | 9         | 3       | 212       | 16,725 |
| Steelhead trout | 47                                     | 76/80                  | 86  | 10      | 3       | 0         | 1       | 72        | 5,475  |

(a) Exposure periods varied because of differential mortalities; thus on day 40 there were no chinook salmon remaining in the cage.

(b) Numerator = number infected. Denominator = number examined.

Table 3. Incidence and intensity of glochidial infection in native chinook salmon, coho salmon, cutthroat trout, and steelhead trout collected near river mile 21 in the Siletz River from May 22 to June 19, 1971.

| Date   | Chinook salmon         |                        | Coho salmon            |                        | Cutthroat trout        |                        | Steelhead trout        |                        |
|--------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
|        | Incidence <sup>a</sup> | Intensity <sup>b</sup> |
| May 22 | -                      | -                      | 6/8                    | 4/29                   | -                      | -                      | -                      | -                      |
| 24     | 2/2                    | 276/553                | 1/1                    | -/107                  | -                      | -                      | -                      | -                      |
| 28     | 1/1                    | -/1,606                | 6/6                    | 47/281                 | -                      | -                      | -                      | -                      |
| 30     | 5/5                    | 421/2,104              | 2/2                    | 42/85                  | -                      | -                      | -                      | -                      |
| June 1 | 1/1                    | -/76                   | 7/7                    | 56/389                 | -                      | -                      | -                      | -                      |
| 3      | 3/3                    | 385/1,155              | -                      | -                      | -                      | -                      | -                      | -                      |
| 4      | 59/59                  | 362/21,337             | 19/21                  | 6/130                  | -                      | -                      | 35/35                  | 118/4,121              |
| 7      | 1/1                    | -/2,288                | -                      | -                      | -                      | -                      | -                      | -                      |
| 11     | -                      | -                      | -                      | -                      | 1/1                    | -/102                  | -                      | -                      |
| 19     | 2/4                    | 100/400                | 2/5                    | 0.4/2                  | -                      | -                      | 13/16                  | 5/82                   |
| Totals | 74/76                  | 399/29,519             | 43/50                  | 24/1,023               | 1/1                    | -/102                  | 48/51                  | 88/4,203               |

(a) Numerator = number infected. Denominator = number examined.

(b) Numerator = mean number per fish. Denominator = total number.

ranged in length from 40.5 to 69.5 mm with a mean of 52.0 mm, was 29,519 (Table 3), and their mean numbers were 368 and 625, respectively. Of the native fish, 32% had less than 100 parasites, 13% had 100 to 250, 25% had 251 to 500, 25% had 501 to 1,000, and 5% had more than 1,000 parasites. The highest infection intensities found in a captive and a native chinook salmon, both of which were dead, were 2,894 (2X) and 2,288 (2X) parasites, respectively. This is in contrast with 1,695 and 1,606 (2X) parasites found in a captive and a native fish that were alive and appeared healthy.

Captive cutthroat trout had the second highest infection intensity. The total and mean number of parasites present in 79 of 90 live captive fish collected during their 55-day exposure period were 16,725 and 212, respectively (Fig. 5, Table 2). Only one native cutthroat trout was captured. This fish was 185.5 mm in fork length and was infected with 102 parasites (Table 3). The highest number of parasites found in a cutthroat trout, which was moribund, was 2,212 (2X), contrasted with 1,420 parasites in another captive fish that had been actively swimming in the cage.

The total and mean number of parasites present in 76 of 80 live captive steelhead trout sampled during their 47-day exposure period were 5,475 and 72, respectively (Fig. 5, Table 2). The total and mean number of parasites present in 48 of 51 live native fish were 4,203 and 88, respectively (Table 3). Of these fish, 74% had less

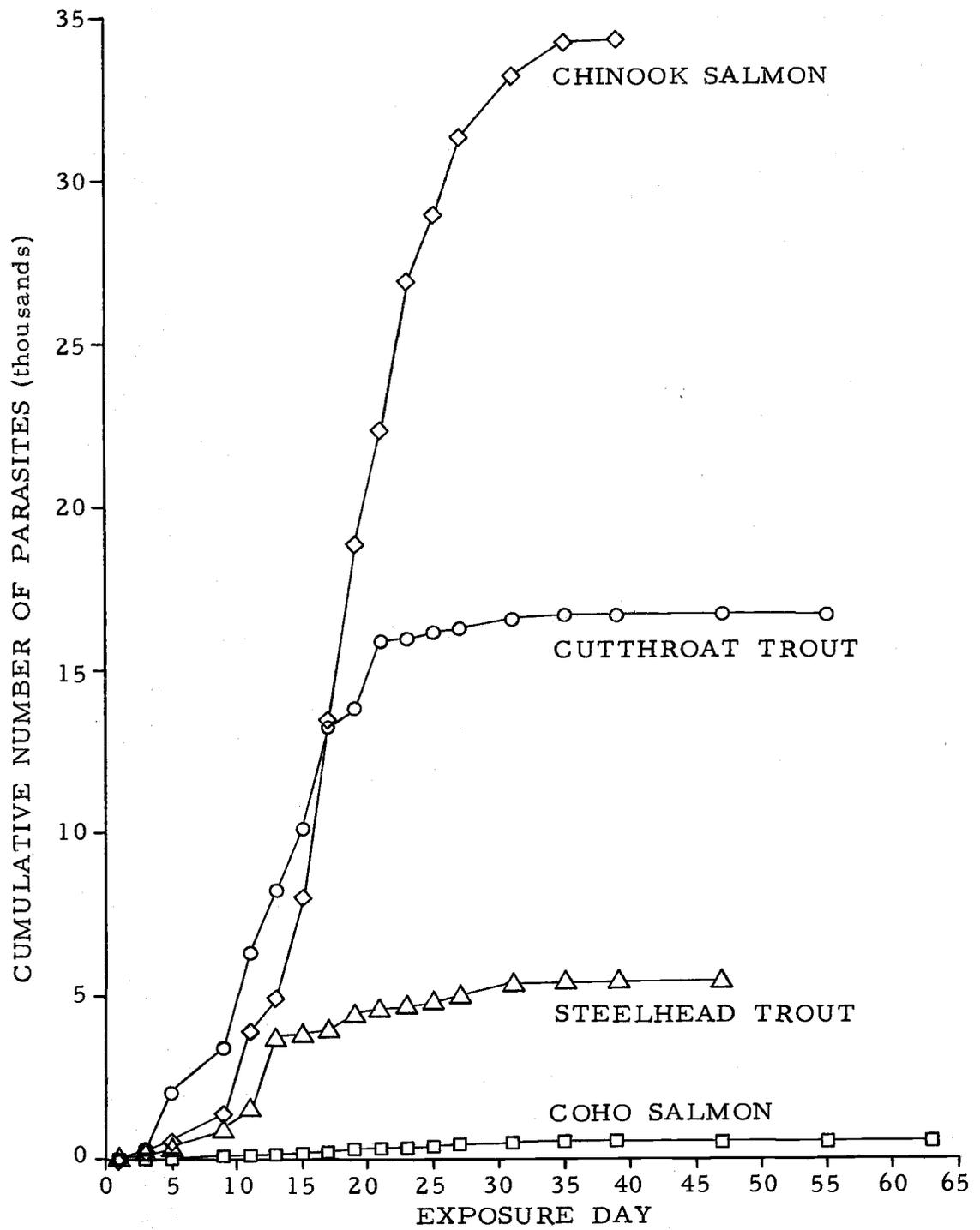


Figure 5. Cumulative infection levels in four salmonid species exposed continuously to the glochidia of Margaritifera margaritifera.

than 100 parasites, 20% had 100 to 250, and 6% had 251 to 500 parasites. The native fish ranged in fork length from 31.0 to 42.0 mm and averaged 35.5 mm. The highest individual infections observed in a captive and a native fish were 2,016 (2X) and 439 parasites, respectively. Both fish were alive, but the captive fish was very lethargic.

Coho salmon had the lowest incidence and infection intensity of the captive and native salmonids (Tables 2, 3). The total and mean number of parasites present in 71 of 95 live captive fish sampled during their 63-day exposure period were 576 and 8, respectively (Fig. 5, Table 2). All of these fish had less than 50 parasites. The total and mean number of parasites present in 43 of 50 live native fish were 1,023 and 24, respectively (Table 3). These fish ranged in fork length from 30.5 to 69.5 mm and averaged 46.0 mm. Of these fish, 94% had less than 100 parasites, and the remaining 6% had less than 200 parasites. The highest individual infection intensity was 162 parasites.

Approximately 65% of the parasites in captive coho salmon were encysted in the gill rakers, and the remaining 35% were in the gill filaments and arches. The reverse relationship was found for the native coho salmon and the other native and captive species. Their gill filaments were more heavily infected than the gill rakers.

The total and mean number of parasites present in 5 of 5

threespine sticklebacks examined, which averaged 63.0 mm in fork length, were 1,122 and 224, respectively. The highest individual infection intensity was 495 parasites observed in the gills of a dead fish.

Native prickly sculpins were relatively resistant to glochidial infection. A total of 8 parasites was attached to, but not completely encysted in, the gills of 3 of 11 fish, which averaged 77.0 mm in fork length. Also, 21 unattached glochidia were present in a mucus secretion located between the hemibranchs or the gill filaments of these fish.

There was no difference in infection levels between the right and left holobranch sets of the live native salmonids. Parasite counts for the right and left holobranchs, respectively, were: 12,323 and 12,902 for chinook salmon; 514 and 495 for coho salmon; 49 and 53 for cutthroat trout; and 2,053 and 2,150 for steelhead trout. The same comparison was not made for the captive fish because of insufficient time.

### Effects of Glochidiosis on Captive Fish

#### Relationship Between Condition Factor and Infection Intensity

Two different relationships were found when infection intensities and condition factors were compared. A negatively sloped line

described the relationship for chinook salmon. Fish with the heaviest infections had the lowest condition factors (Fig. 6); whereas, the reverse relationship was found for cutthroat trout (Fig. 7). Also, at the higher infection levels the trout were in better condition than the salmon. For the salmon and trout, 7 and 35%, respectively, had condition factors above 0.9.

A line was not fitted to the data for steelhead trout because there was no apparent relationship (Fig. 8). These fish were also in better condition than the chinook salmon; 15% of them had condition factors above 0.9. The infection levels in coho salmon were too low to make any comparison between condition and infection intensity.

I can only speculate as to the reason for the above contrary relationships. Compared with the other species, the chinook salmon had the highest infection intensities and this may have accounted for their poor condition. Perhaps if the other fish species had higher numbers of parasites their condition may also have been affected adversely. This hypothesis could be tested experimentally by infecting fish in the laboratory with known numbers of parasites.

### Mortalities

Exposure periods for the captive fish varied because these species had different mortalities. Total deaths were: 203 for chinook salmon, 199 for steelhead trout, 189 for cutthroat trout, and

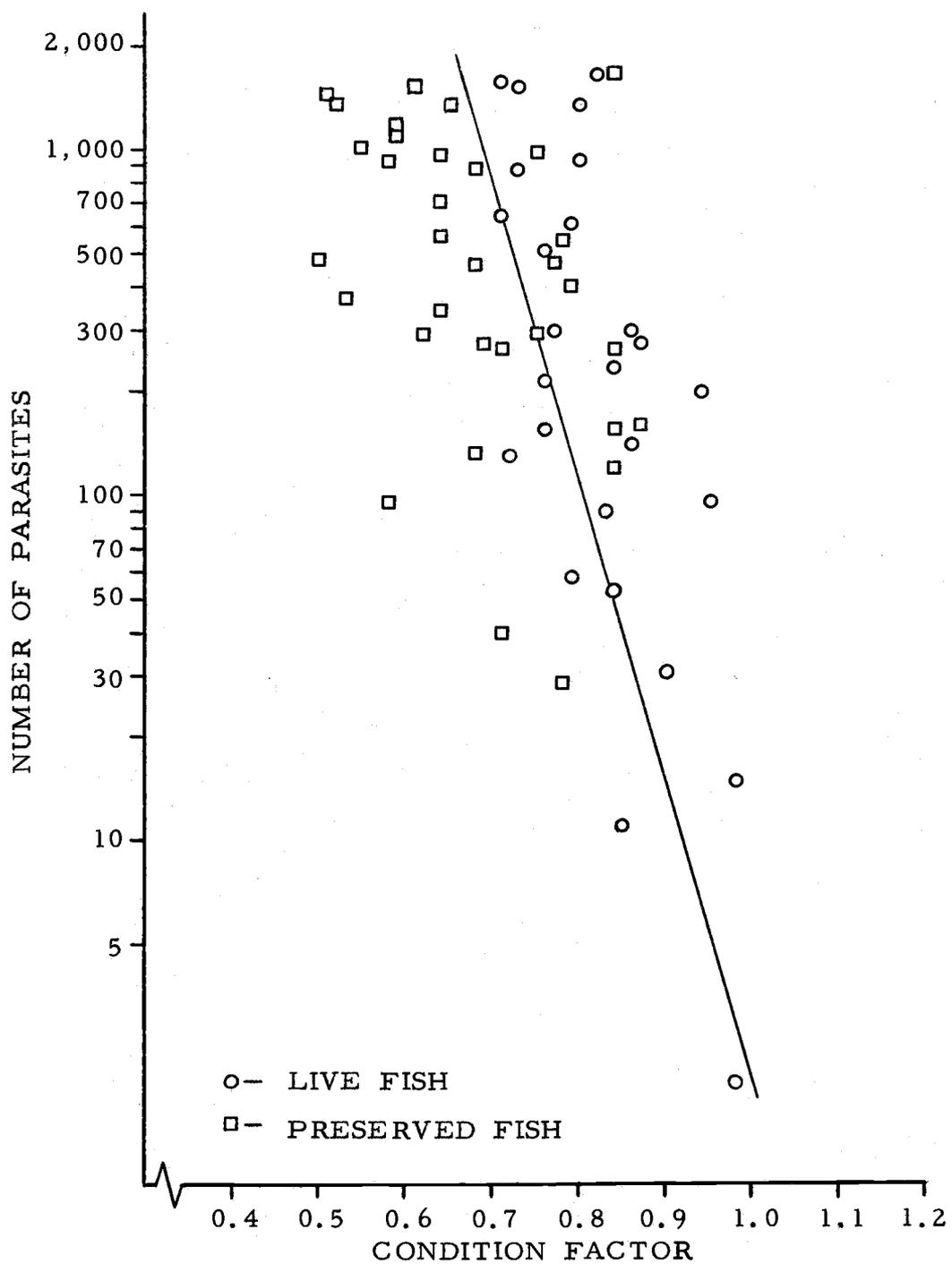


Figure 6. Relationship between intensity of glochidial infection and condition factors for chinook salmon.

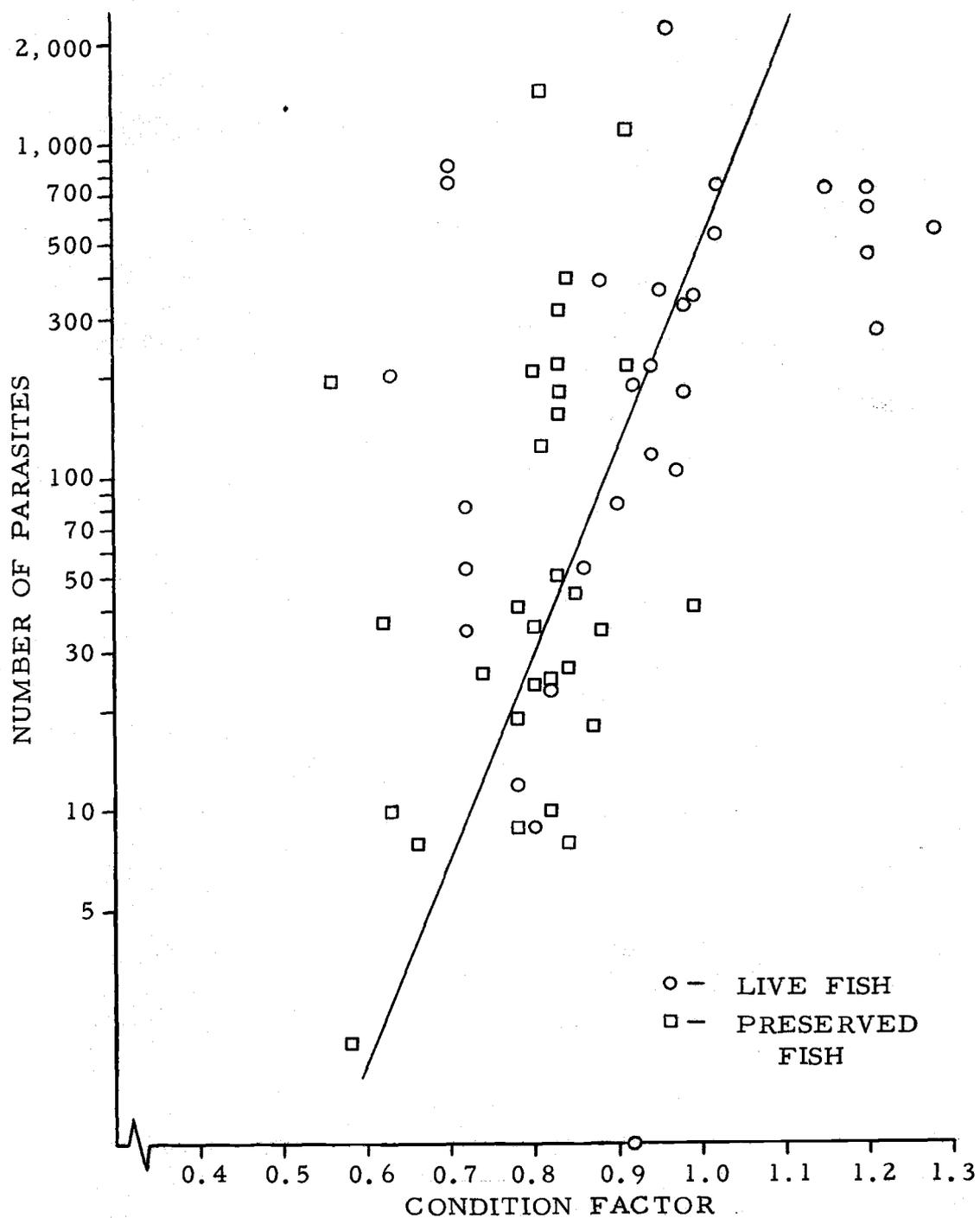


Figure 7. Relationship between intensity of glochidial infection and condition factors for cutthroat trout.

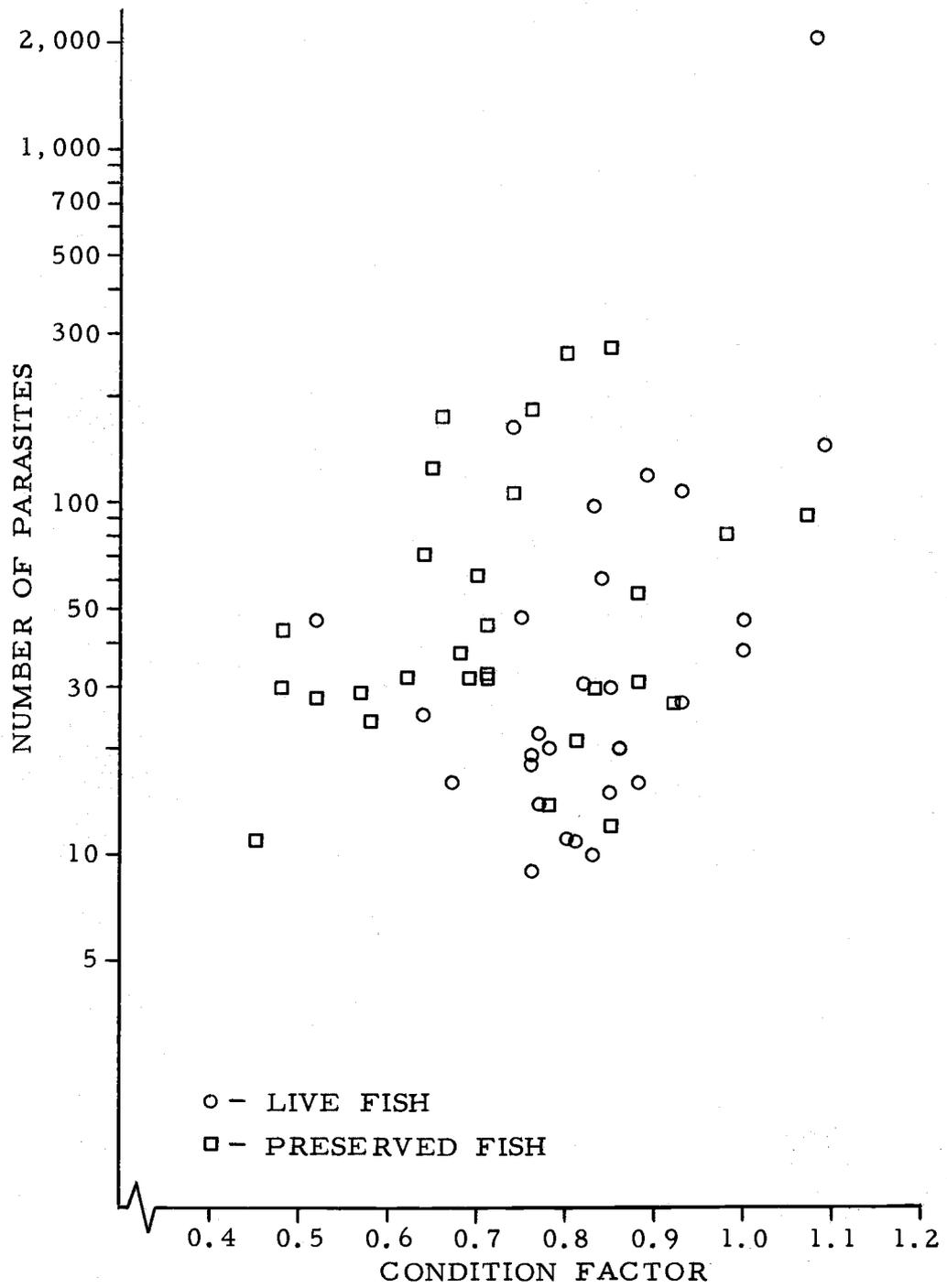


Figure 8. Relationship between intensity of glochidial infection and condition factors for steelhead trout.

177 for coho salmon (Table 4). However, many of these fish were not examined for parasites because of decomposition.

The highest mean infection intensity was 660 parasites in 103 of 105 infected chinook salmon (Table 4). Fungal infection of the gills occurred more frequently on these fish than on the gills of the other species (Table 4). The highest number of daily deaths was 14, which occurred on day 21 (Fig. 9). During the last 26 days of exposure the mean daily mortality was approximately 7 fish.

The second highest infection intensity was observed in 41 of 48 infected cutthroat trout. These fish had an average of 309 parasites (Table 4). They had fewer fungal infections than chinook salmon but more than steelhead trout and coho salmon. The mortality for the cutthroat trout was bimodal. The maximum numbers of daily deaths, which were 16 and 8, occurred on day 13 and 47, respectively (Fig. 9).

The lowest infection intensities were found in steelhead trout and coho salmon. Of the trout, 38 of 40 were infected with an average of 14 parasites, and 24 of 58 of the salmon were infected with an average of 10 parasites (Table 4). Their daily mortalities were less than five during the first 31 days of exposure. Thereafter, the daily mortalities of steelhead trout and coho salmon increased to maximums of 24 and 9, respectively (Fig. 9).

Metacercariae of the "salmon poisoning" trematode Nanophyetus

Table 4. Incidence and intensity of glochidial and fungal infections in dead captive fish.

| Species         | Total no. dead | Incidence <sup>a</sup> | Glochidial Infections                          |         |         |          |       | Parasites |        | Fungal Infections |                                  |                                  |
|-----------------|----------------|------------------------|--|---------|---------|----------|-------|-----------|--------|-------------------|----------------------------------|----------------------------------|
|                 |                |                        | Percent of fish with the following intensities |         |         |          |       |           |        | Percent of fish   |                                  |                                  |
|                 |                |                        | <100   | 100-200 | 251-500 | 501-1000 | >1000 | Mean      | Total  | Uninfected        | With fungi on 1 to 4 holobranchs | With fungi on 5 to 8 holobranchs |
| Chinook salmon  | 203            | 103/105                | 12   | 13      | 24      | 30       | 21    | 660       | 68,011 | 18                | 52                               | 30                               |
| Coho salmon     | 177            | 24/58                  | 100  | 0       | 0       | 0        | 0     | 10        | 251    | 91                | 9                                | 0                                |
| Cutthroat trout | 189            | 41/48                  | 52   | 6       | 21      | 15       | 6     | 309       | 12,683 | 39                | 42                               | 19                               |
| Steelhead trout | 199            | 38/40                  | 100  | 0       | 0       | 0        | 0     | 14        | 516    | 88                | 10                               | 2                                |

<sup>a</sup>Numerator = number infected. Denominator = number examined. Some fish were not examined because of decomposition.

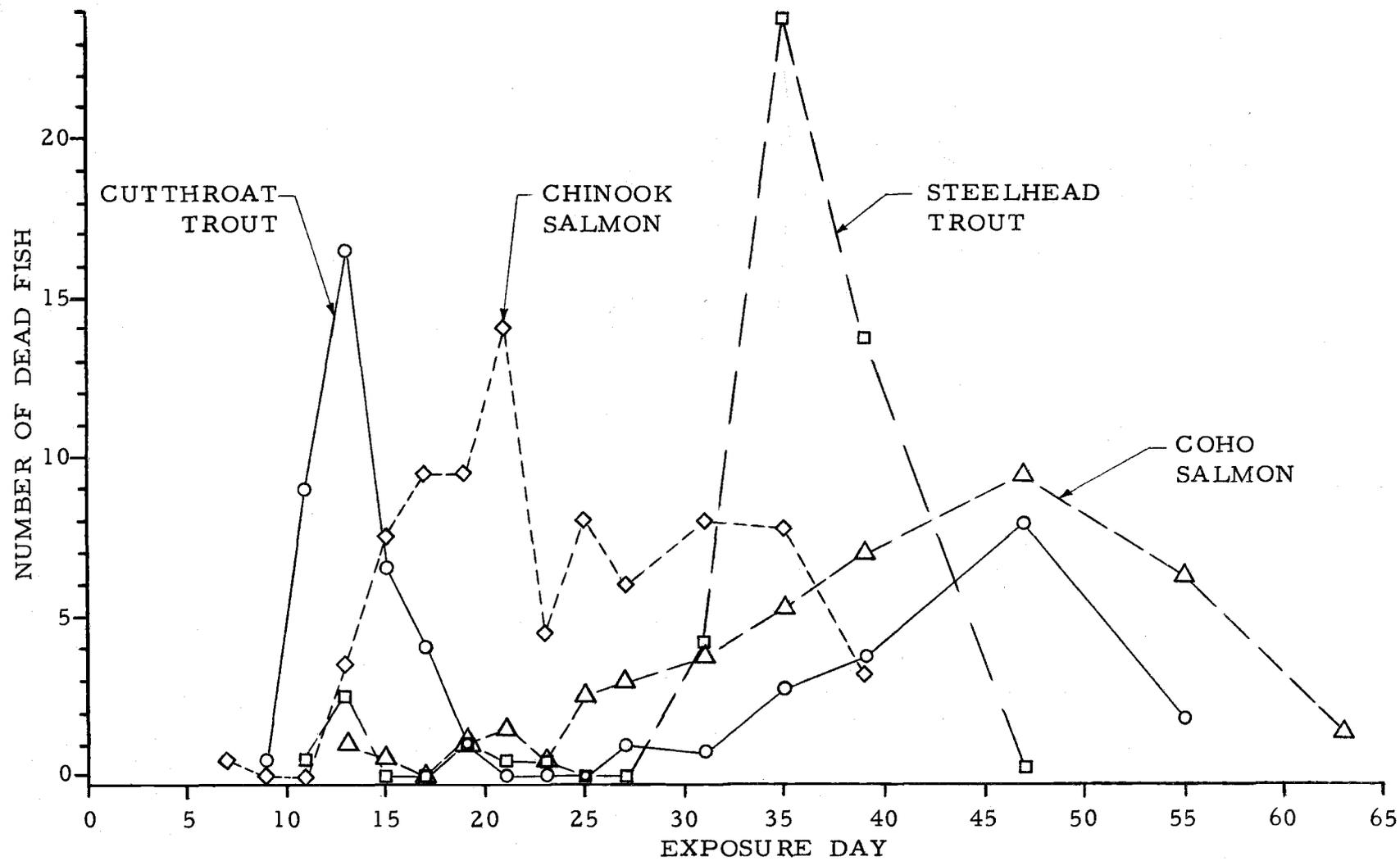


Figure 9. Daily mortalities of four salmonid species exposed continuously to the glochidia of Margaritifera margaritifera.

salmincola were found encysted in the gills of some of the dead captive fish. Infected fish were found on exposure days 35 to 55. The number of metacercariae per fish was usually less than 50. The maximum observed numbers, which were approximate, were: 150 for chinook salmon, 500 for cutthroat trout, 300 for steelhead trout, and 200 for coho salmon.

The percentages of the dead captive fish without food in their stomach or intestine were: 25% for chinook salmon, 46% for cutthroat trout, 58% for steelhead trout, and 65% for coho salmon.

Apparently starvation was the major cause of death of steelhead trout and coho salmon because these fish did not have heavy glochidial, fungal, or metacercarial infections (Table 4). Furthermore, 86 and 80% of the steelhead trout and coho salmon deaths, respectively, occurred after exposure day 31 when glochidia were no longer being released by the mussels.

Of the chinook salmon and cutthroat trout, 78 and 48%, respectively, of their total mortalities occurred before day 31. These fish probably died because of the heavy glochidial and fungal infections (Table 4). All moribund chinook salmon and cutthroat trout removed from the cages had fungal infections in addition to heavy glochidial infections. After exposure day 31, starvation may have contributed to the mortalities.

## Metamorphosis and Histopathology

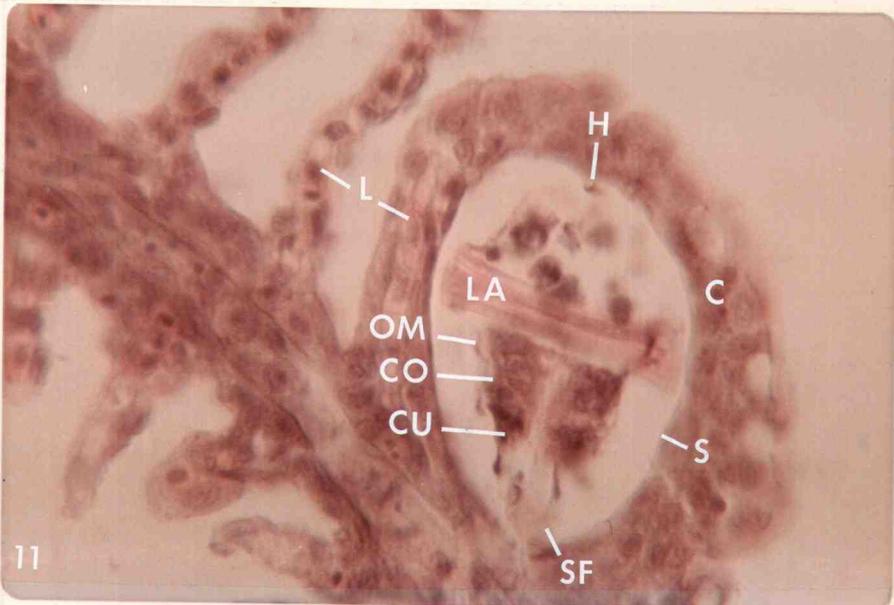
Glochidia were observed undergoing metamorphosis on the gills of captive and native chinook salmon, coho salmon, cutthroat trout, and steelhead trout, and also on native threespine sticklebacks. All of the salmonids were alevins, except for the native cutthroat, which was an adult.

Harms (1907, 1909) found that M. margaritifera glochidia were exclusively gill parasites on cyprinids in Europe; whereas, Baer (1951) stated that they were always found on the fins of cyprinids. My observations are in agreement with Harms; however, due to insufficient time, a thorough examination of fin tissue was not performed. A brief survey of the external surfaces of several heavily infected chinook salmon revealed three cysts located between the rays of the pectoral and caudal fins. These cysts were not sectioned; therefore, their confirmation as M. margaritifera can not be established. Matteson (1948) found that the hookless glochidia of E. complanatus were often fastened to fish fins and scales, but none were observed to survive through metamorphosis.

In the following discussion the term parasite refers to the glochidium, but not the cyst wall, which surrounds the parasite's shell and is of host origin. The term cyst or encysted glochidium refers to the parasite including the cyst wall.

Histological sections were made from the gills of the four species of captive salmonid fish, and there were no observed differences in glochidial development in these fish. Encysted glochidia were found on the gill filaments, arches, rakers, and occasionally on the pseudobranchs of all captive fish species. Except in coho salmon, they were found most often on the gill filaments where encystment occurred on the lamellae or on the side of the filament. Usually, encystment involved only one lamella. Lefevre and Curtis (1912) found that L. ligamentina glochidia encysted most often on the gill filaments, and that the distal end of the filaments were more heavily infected. The gill lamellae are covered by simple squamous epithelium, and each lamella contains numerous capillary beds. Cysts on the side of the gill filament are embedded in a thin cuboidal or squamous epithelium, which also covers the afferent and efferent filamental arteries. The cyst walls of glochidia located in the stratified cuboidal epithelium of the gill arches and rakers were more compact and thicker than those of glochidia on the filaments.

Glochidia recently encysted in gill filaments have uneven walls averaging 15  $\mu$  in thickness (Fig. 10, 11). The walls of these glochidia are formed by accumulated host epidermal cells, and one lamella from each side may also be fused to the wall. At this stage, the glochidia have not increased in size or undergone metamorphosis. The larval adductor muscle, mantle, enclosed host tissue, and shell



Figures 10 and 11. Photomicrographs of cross sections of recently encysted Margaritifera margaritifera glochidia in the gill filaments of chinook salmon. L = lamellae, C = cyst wall, S = shell, H = hinge ligament, LA = larval adductor muscle, OM = outer mantle, CO = columnar cells of inner mantle, CU = cuboidal cells of inner mantle, HT = host tissue, SF = shell flange, which is out of focus in these photomicrographs. X 700.

flanges were observed in cross sections of the glochidium (Fig. 10, 11). Young (1911) and Lefevre and Curtis (1912) also observed these structures in L. ligamentina glochidia encysted in fish gills. Spaces commonly occur within the parasite where glochidial tissue has separated from the calcareous shell undoubtedly as a result of fixation.

As first observed by Arey (1932b), the larval mantle is composed of outer and inner cell layers. The outer layer consists of simple squamous cells that are located next to the shell (Fig. 10, 11) and which form the definitive mantle (Fig. 12). The inner layer consists of columnar cells, 10 by 3  $\mu$ , and cuboidal cells, 5  $\mu$  in size, located dorsally and ventrally, respectively, on the mantle (Fig. 10, 11). The cytoplasm of the cells of the inner layer is darkly basophilic and contains granules. Arey (1932b) inferred that these granules are associated with enzymatic digestion. Host tissue is pinched between the two shell flanges, which are located on the ventral edge of the shell, and both the host tissue and flanges are in contact with the cuboidal cells of the inner mantle.

When the parasite is about 120  $\mu$  in cross-sectional height, two to four gill lamellae from each side are usually enclosed within the cyst wall, and epidermal cells fill the spaces between the lamellae (as shown in Fig. 13 for a later developmental stage). Except for the lamella that is grasped by the glochidium, those that become part of

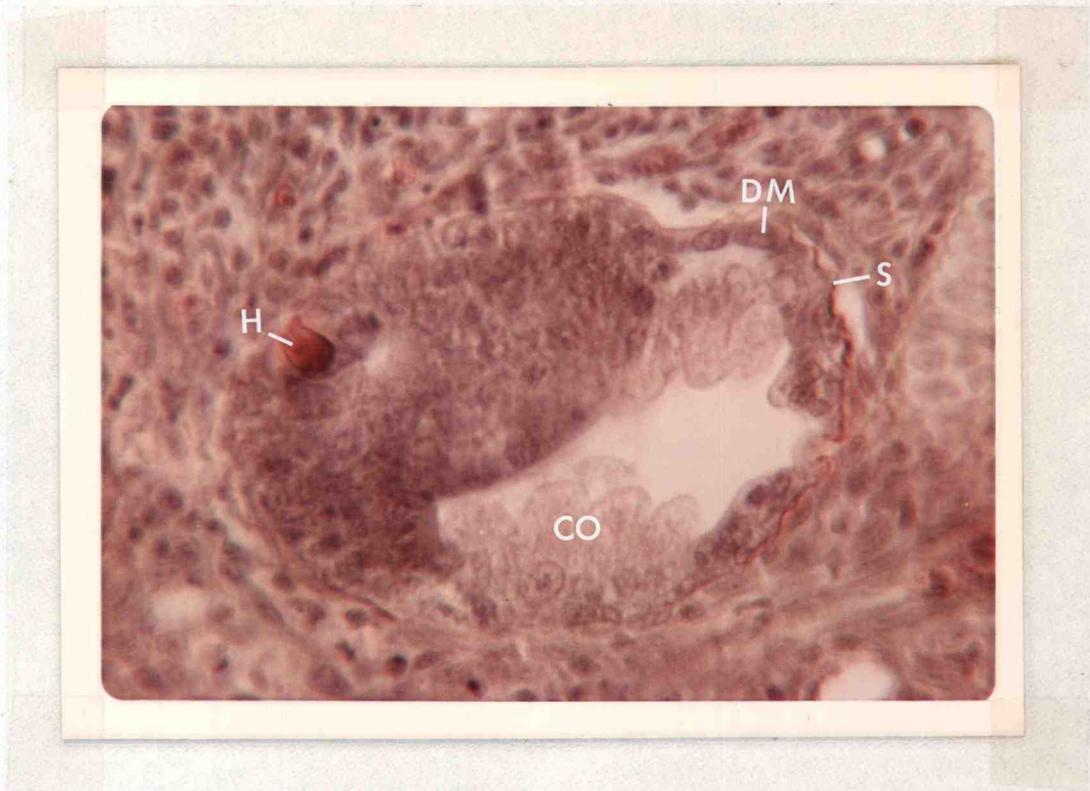


Figure 12. Photomicrograph of a cross section of a Margaritifera margaritifera glochidium encysted in the gill filament of a chinook salmon. The parasite measures approximately 120  $\mu$  in height. S = shell, H = hinge ligament, DM = definitive mantle, CO = columnar cells of inner (larval) mantle, which have increased in size. X 700.

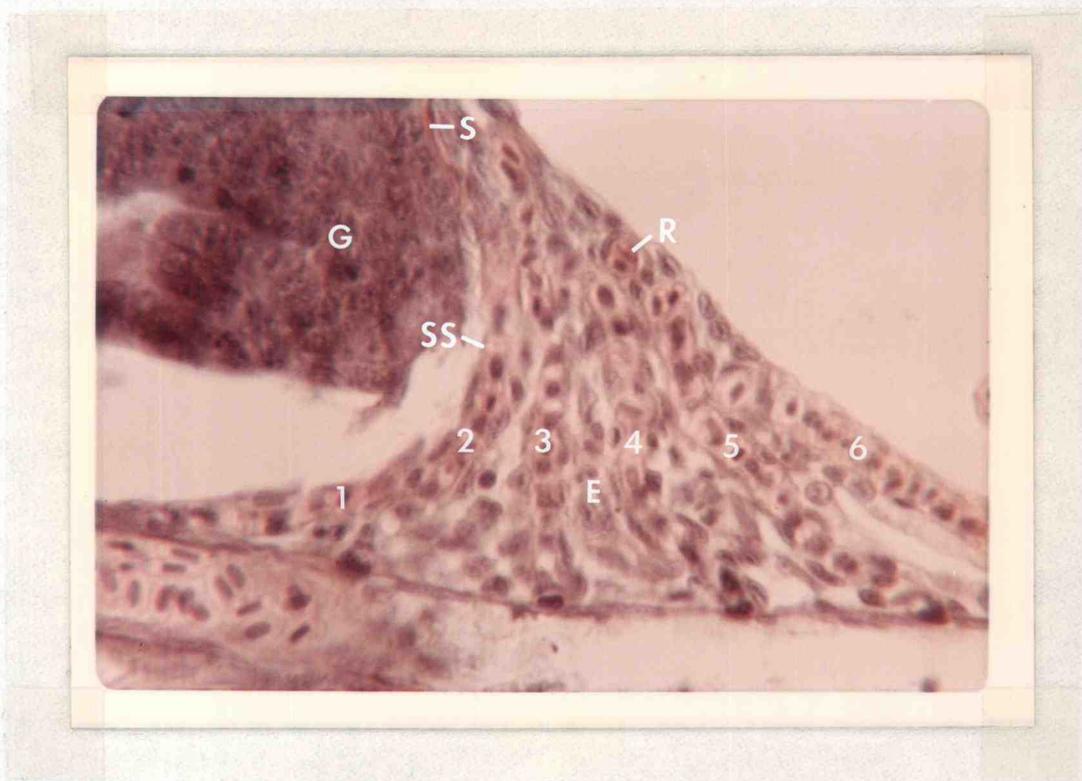


Figure 13. Photomicrograph of a cross section of one side of the cyst wall of a *Margaritifera margaritifera* glochidium in the gills of a chinook salmon. The wall is composed of six gill lamellae (labeled 1 through 6) and epidermal cells (E). G = glochidium, S = shell, R = red blood cells, SS = simple squamous epithelium of the second lamella. X 700.

the cyst wall retain their structure and appear undamaged. Red blood cells are present in the capillary beds of these lamellae (see Fig. 13); therefore, blood apparently continues to flow in these vessels. Host tissue pinched by the glochidium and also the larval adductor muscle have been broken down and apparently are absorbed by the parasite. A prominent hinge ligament and definitive mantle can be seen in cross sections of this developmental stage (Fig. 12). Also, the columnar cells of the inner mantle have increased in size and now take a lighter basophilic stain than formerly (Fig. 12). According to Young (1911) and Arey (1932b), these cells function in the absorption and digestion of the larval and host tissues. The entire cyst is covered by squamous epithelium, and the outer part of the cyst wall, i. e., the part of the cyst that is not embedded in and surrounded by gill lamellae, has become thinner and averages about  $8 \mu$  in thickness.

When the parasite is approximately  $210 \mu$  in height, usually five gill lamellae from each side are enclosed within the cyst wall (Fig. 13). But, when cysts are close together there are fewer lamellae per cyst. At least the first two lamellae on each side take the shape of the nearly round parasite (Fig. 13). The inner lamella lies immediately beside the parasite's shell, and only a simple squamous cell layer separates the lamellar capillary beds from the shell (Fig. 13). Blood apparently continues to flow in these

capillaries because they contain red blood cells. A squamous layer about two to three cells thick covering the outer part of the cyst, the definitive foot and mantle, and anlage of the intestinal tract within the mesosomal area of the parasite can be seen in cross sections of this developmental stage (Fig. 14).

Clubbing of the gill filament results when one or more large cysts are located near the end of the filament (Fig. 15). Lamellae from the parasitized side of the filament are enclosed within the cyst wall and those on the other side are fused to each other (Fig. 15). The tip of the filament may bend around and fuse to the cyst wall (Fig. 15). Large cysts also displace lamellae of adjacent gill filaments.

Parasites larger than  $210\ \mu$  were observed on sectioned gill tissue from all captive fish species. Large parasites occurred more frequently on chinook salmon and cutthroat trout than on coho salmon and steelhead trout. Lengths and widths, excluding the cyst wall, of the largest parasites for each species were approximately:  $270$  by  $160\ \mu$  for chinook salmon,  $265$  by  $200\ \mu$  for coho salmon,  $350$  by  $250\ \mu$  for cutthroat trout, and  $275$  by  $165\ \mu$  for steelhead trout. Length was measured from frontal or sagittal sections, and the width from the widest point perpendicular to the length measurement. Usually, the parasite body is withdrawn from the calcareous shell presumably due to fixation. The shell normally remains attached to the cyst wall.

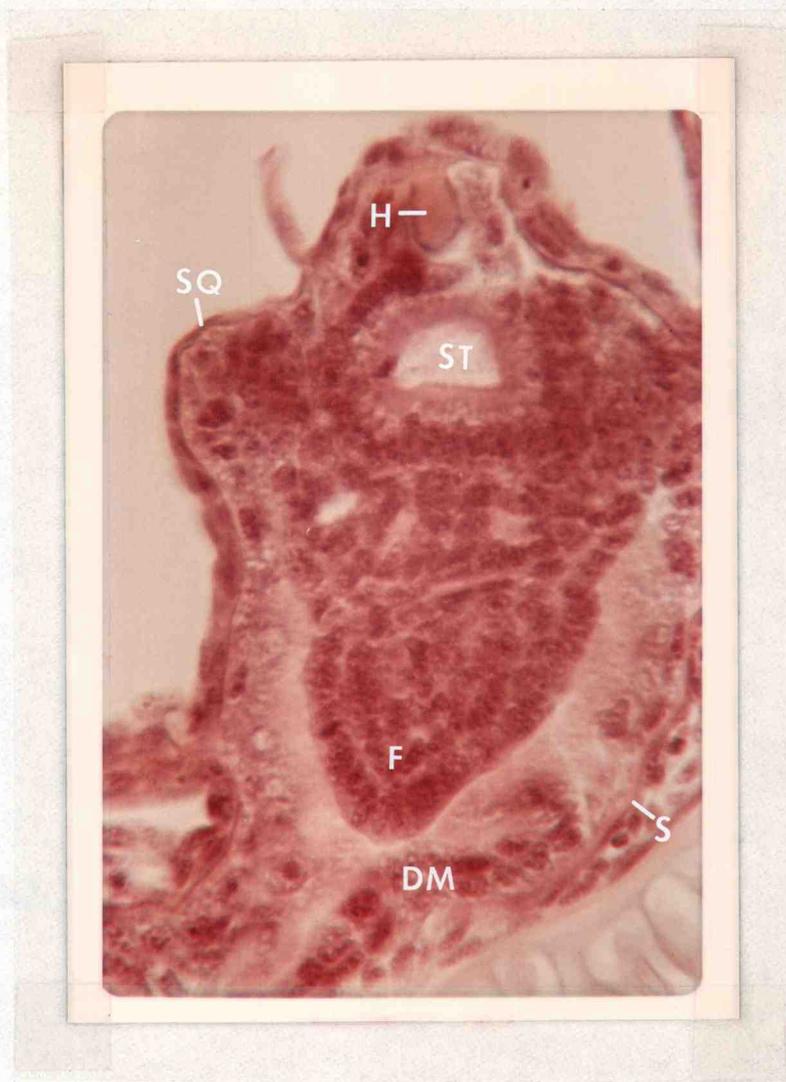


Figure 14. Photomicrograph of a cross section of a Margaritifera margaritifera glochidium encysted in the gill filament of a chinook salmon. The parasite measures approximately 210  $\mu$  in height. S = shell, H = hinge ligament, F = foot, DM = definitive mantle, ST = stomach in mesosomal area, SQ = squamous layer of cyst wall, this layer is several cells in thickness and covers the outer part of the cyst. X 700.

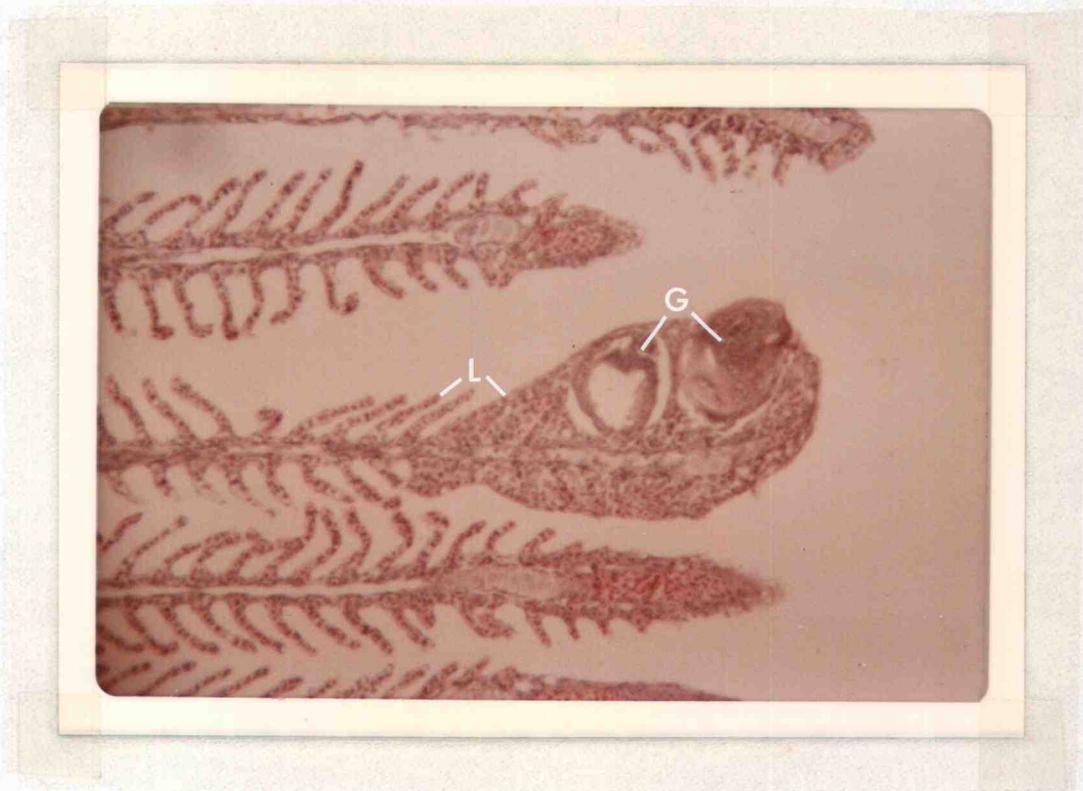


Figure 15. Photomicrograph of a frontal section of chinook salmon gill filaments showing clubbing that resulted from fusion of lamellae (L) to the cyst walls of Margaritifera margaritifera glochidia (G). X 158.

The outer part of the cyst wall of parasites measuring over 240  $\mu$  in length is covered by squamous epithelium, which is now only one or two cells in thickness. The basal or inner part of the cyst wall may be composed of 10 to 15 fused gill lamellae. This inner part of the cyst wall is now thinner because of a reduction in the number of epidermal cells between the lamellae. However, sloughing of these cells was not observed.

Previous workers who studied the histopathology of glochidiosis caused by species of mussels other than M. margaritifera did not report such extensive lamellar fusion in cyst formation. Their glochidia do not increase in size during metamorphosis and thus lamellar fusion may be limited. Lefevre and Curtis (1912) observed, by microscopic examination of intact gill tissue only, the "smoothing" or obliteration of gill lamellae near the cysts of L. ligamentina on the gills of largemouth bass. Microscopic examination of sectioned tissue may have revealed fusion. Arey (1932a) stated that lamellae adjacent to the attached glochidia of Lampsilis anodontoides and L. luteola may fuse to form the basal part of the cyst, but he did not give the number of lamellae involved.

I found, as Young (1911) also observed, the walls of fully-formed cysts consist almost entirely of epithelial cells. Arey (1932a) reported that the cysts of L. luteola, whose glochidia are approximately three times larger than M. margaritifera glochidia, consisted

of epithelial cells and connective tissue. Apparently larger glochidia "bite" deeper into the host tissue, and the resultant cyst is embedded in the stromal layer.

There are two contradicting hypotheses on cyst formation. Young (1911) and Lefevre and Curtis (1912) maintained that cysts resulted from mitotic cell division; whereas, Arey (1932a) stated that they were formed by cell migration. I found only five clear mitotic figures in sections of approximately 200 cysts. However, because all these cysts had already formed, mitotic cell division may not be eliminated as an important mechanism in early cyst formation. This question could be resolved by infecting fish experimentally and then sectioning the infected gill tissue at periodic intervals after attachment of the glochidia.

After the parasite exceeds 240  $\mu$  in length, it is essentially a miniature adult. An almost completely formed digestive system of stomach, digestive gland, and intestine, a pair of adductor muscles connected to the shells, a rudimentary nervous system, and a ciliated foot possessing protractor and retractor muscles can be seen in sagittal sections of this developmental stage (Fig. 16). In addition to these structures, gill buds and a hemocoel that has developed within the definitive mantle can be seen in cross sections (Fig. 17). The large cells of the larval mantle, which were formerly columnar, are still present on the inner mantle layer (Fig. 18). Arey (1932b)

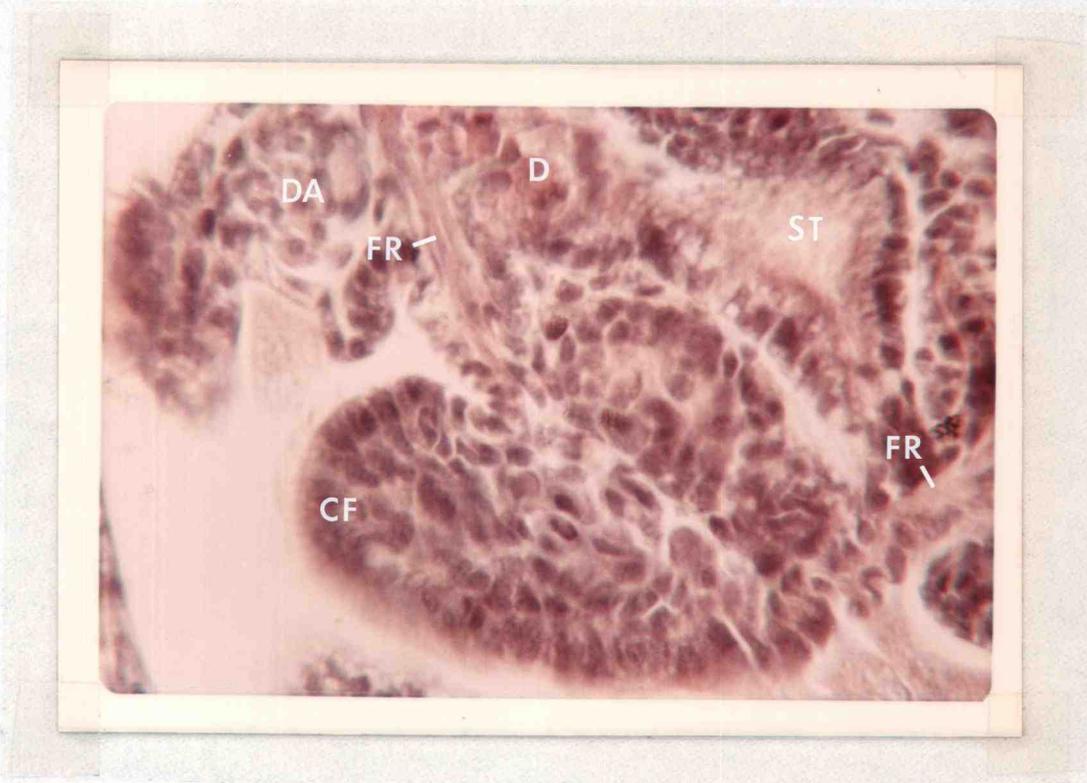


Figure 16. Photomicrograph of a sagittal section of a Margaritifera margaritifera juvenile that had nearly completed metamorphosis. The parasite was encysted in the gills of a steelhead trout. CF = ciliated foot, DA = definitive adductor muscle (cross sectioned), FR = foot retractor muscles, D = digestive gland, ST = stomach. X 700.

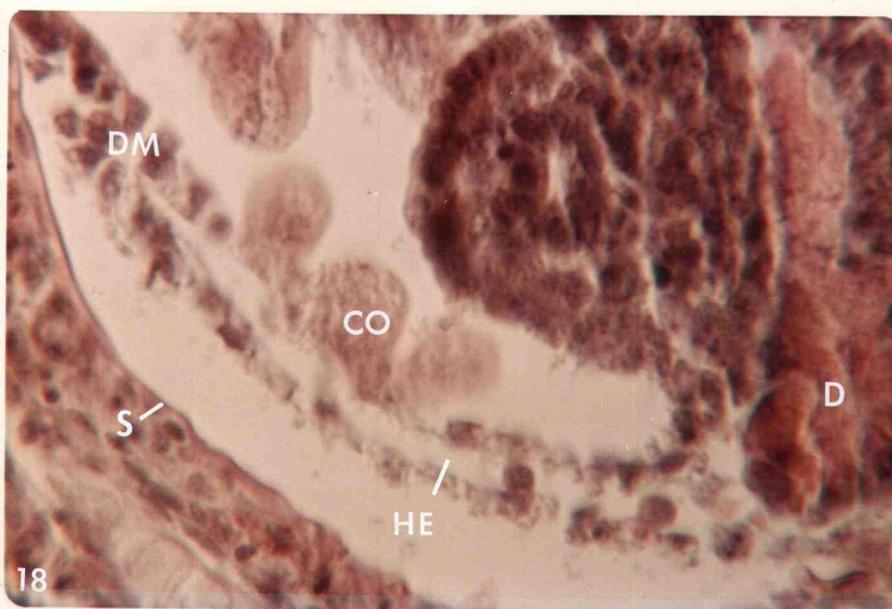
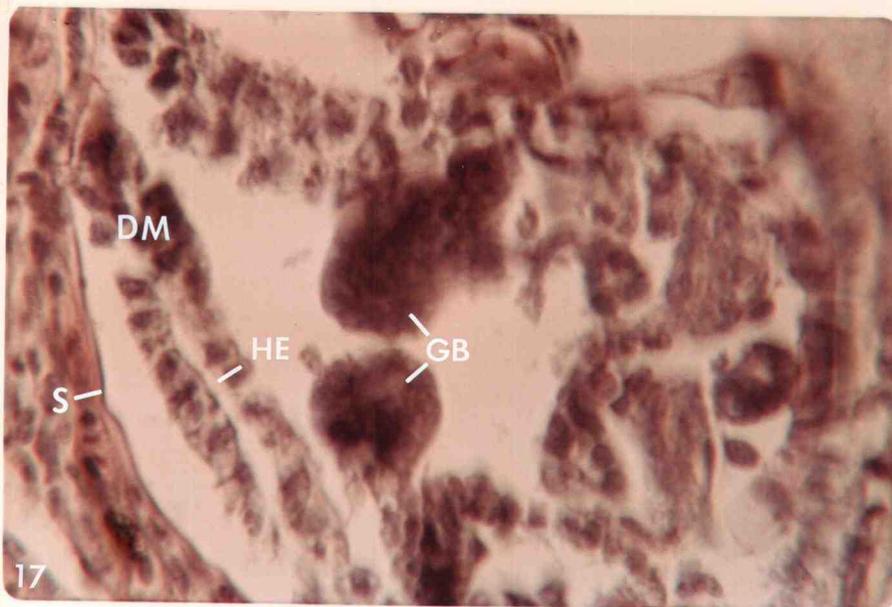


Figure 17 and 18. Photomicrographs of cross sections of a Margaritifera margaritifera juvenile that had nearly completed metamorphosis. The parasite was encysted in the gills of a coho salmon. S = shell, DM = definitive mantle, HE = hemocoel, GB = gill buds, D = digestive gland, CO = former columnar cells of larval mantle that have increased in size and become mushroom-shaped. X 700.

found that these mushroom-shaped larval cells were still present on free-living juveniles of L. anodontoides and L. luteola.

Whether or not the parasite utilizes for nutritional purposes its close association with the lamellar capillary beds of the fish could not be determined from histological sections. Since the glochidia of M. margaritifera, unlike the glochidia of some other mussel species, do grow during their parasitic stage such a relationship seems likely.

Some sections showed empty cysts with ruptured walls, which presumably resulted from excystation of the glochidia. According to Lefevre and Curtis (1912) and Murphy (1942), when metamorphosis is completed the juvenile mussel breaks the cyst wall with its foot and falls to the stream bottom. The thinning of the wall probably makes it easier for the mussel to leave its host.

Metacercariae of the trematode N. salmincola were observed encysted on some of the gill tissue sectioned from chinook salmon, cutthroat trout, and steelhead trout. The metacercariae usually encysted between the bases of two gill filaments. Lamellae from the gill filaments on each side of the parasite become fused in cyst formation, which apparently is similar to that observed for glochidial encystment. Metacercariae were also found in the blood vessels of the gill filament and encysted in the gill arch musculature.

## DISCUSSION

The difference in susceptibility of the four species of captive fish to glochidial infection could be attributed to differences in: (1) position of the cages such that each species was not exposed to equal numbers of parasites; (2) gill morphology; (3) ventilating rate; (4) behavior; (5) the time of occurrence of parr-smolt metamorphosis; (6) chemical composition of the gill mucus or blood.

Regarding the first possibility, all of the cages were placed at a depth of approximately 1.5 m in an eddy off the main channel where the water depth averaged 3.5 m. The stream bottom was nearly flat, so all of the cages were approximately in the same vertical position during the experiment. The distance between the cages, which were suspended from an overhead cable, was 4.5 m, and the distance between the cages and shore ranged from 3 to 5 m. The water current angled into the steelhead trout and cutthroat trout cages, and then flowed parallel to the overhead cable as it passed first through the coho salmon and then the chinook salmon cages. The cages did not obstruct the flow of glochidia in the water because the chinook salmon were down-current from the other fish and they had the highest infection intensity. For these reasons, I do not believe that the fish were exposed to different numbers of glochidia sufficient to account for the great difference in infection intensity between chinook

and coho salmon. Furthermore, this same spectrum in species susceptibility also occurred with the native fish.

Concerning gill morphology, there were no obvious differences among the four species in the numbers of rakers or filaments on each arch, or in the numbers of lamellae on each filament, or in the size of the rakers or filaments. Also, I observed no differences among the species regarding types of cells or in the thickness of the gill epithelial layers. Therefore, I do not believe that differences in gill morphology can account for the differences in species susceptibility.

I can only speculate on the possible importance of the remaining four possibilities mentioned above. Ventilating rate may affect susceptibility to infection. Paling (1968) used the glochidia of Anodonta cygnea as markers to study water flow over the gills of 27- to 79-cm brown trout. He found that the number of attached glochidia nearly doubled when the respiratory rate of the fish increased from 40 to 75 breaths per minute. I know of no studies on the comparative ventilating rates of salmonid fishes of the size I used. However, even if the rate of chinook salmon exceeded that of the other three species, it is unlikely that this could explain the difference in magnitude of the infection intensities.

Regarding behavior, differences in feeding may have an effect on infection. Baldwin, Millemann, and Knapp (1967) reported that

coho salmon about the same size as mine actively fed on freely-falling cercariae of N. salmincola. Admittedly, the cercariae are larger than the glochidia, 310 to 470  $\mu$  long by 30 to 150  $\mu$  wide compared to 70 by 75  $\mu$  in size, but it is likely that glochidia are visible to fish because I could see them in unshaded water. It seems unlikely that coho salmon would feed less readily than chinook salmon on particles the size of glochidia. However, I know of no study on food particle size preference by salmonids of the length I used.

Another behavioral characteristic to be considered is distribution of the fish in the cage. Even if the distribution was different for each species it is unlikely that this was a factor contributing to the differences in infection intensity because I believe that glochidia are uniformly distributed throughout the water column. This was apparent in the one observation I made on natural release of glochidia from a mussel taken from the river and placed into a bucket of river water. Upon slight agitation of the water the glochidia became evenly distributed. Moreover, Murphy (1942) stated that "since they sink very slowly (3.5 centimeters per minute, with jaws agape) they are easily carried by a slight current."

Hoar (1951), Malikova (1957), and Evropeitseva (1957) found that Atlantic salmon (S. salar) smolts have a lower resistance to injury and disease than parrs. Of the fish I used, chinook salmon was the only species that could have been undergoing parr-smolt

metamorphosis, and whether or not they actually were could not be determined. The other three species usually metamorphose at a larger size, which is attained after one or more years of life in freshwater. Perhaps the physiological changes associated with parr-smolt metamorphosis do lower the resistance of salmonids to glochidiosis, but experimental evidence for this hypothesis is lacking.

Because glochidia cannot swim, they undoubtedly reach the gills passively in the ventilating current. Attachment is probably the result of physical contact with the gills (Murphy, 1942). The tips of adjacent hemibranchs touch during normal respiration (Hughes, 1966), thus facilitating attachment. Differences among fish in the chemical composition of the gill mucus or blood may determine whether attachment is temporary or sufficiently permanent to allow for complete development. These biochemical differences may explain the complete or partial natural resistance of fish to glochidial infection, e. g., as shown by my coho salmon. Murphy (1942) produced heavy M. margaritifera infections in brook trout, Tahoe suckers, "black minnows," and Lahontan reddsides, but only a few of the glochidia survived to complete metamorphosis.

Natural resistance may also have a cellular basis as suggested by Arey's (1932d) work. He studied natural resistance of largemouth bass to L. anodontoides infection. Basophilic spherules, eosinophilic plastids, and eosinophils were present around and within the

glochidia, and the glochidia were either destroyed by cytolysis or sloughed by the second day of infection.

Arey (1932d) also studied acquired immunity of largemouth bass to L. luteola infection and found the same type of cellular infiltration and destruction of glochidia as he observed for largemouth bass with L. anodontoides infection. Development of acquired immunity could be tested experimentally by exposing fish to sublethal numbers of parasites and then subsequently challenging them with a lethal number of glochidia.

This is the first detailed description of the metamorphosis and associated histopathology of M. margaritifera infection. Young (1911), Lefevre and Curtis (1912), and Arey (1924, 1932a, b, c, d) described the metamorphosis and histopathology of infections caused by Lampsilis recta, L. ligamentina, L. luteola, L. anodontoides, and Unio complanatus on the gills of freshwater fishes. The glochidia of these species, however, do not increase in size during metamorphosis as do the glochidia of M. margaritifera. The histopathology caused by these two types of glochidia although basically similar differs in some details as previously mentioned.

Salmonid gills form an intricate sievelike structure through which water flows (Randall, 1970), and the lamellae represent the main respiratory portion of the gill (Lagler, Bardach, and Miller, 1962). Randall (1970) reported that a reduction of blood flow in a

lamella may be compensated for either by an increase in blood flow through other lamellae since all blood pathways are not fully utilized, or by the shunting of blood through alternate nonrespiratory capillary beds within the filament. Such reduction or complete cessation of blood flow undoubtedly occurs in the lamella grasped by a glochidium. Thus, one or both of the above compensatory mechanisms is probably operative during infections. However, in heavy infections of 12 to 15 glochidia such mechanisms may become ineffective for respiration.

The glochidia of M. margaritifera are often encysted on the side of the gill filament in the epidermal tissue that covers the afferent and efferent filamental arteries. This tissue is approximately 10 to 15  $\mu$  in thickness; therefore, the attached glochidium, which is 70 by 75  $\mu$  in size, undoubtedly constricts the blood flow in these arteries. Similarly, Arey (1924, 1932a) found that the hookless glochidia of L. luteola cut through the epithelium and pinched the filamental blood vessels. Matteson (1948) also reported that the hookless glochidia of E. complanatus pinched the filamental arteries so that the blood flow was stopped. In heavy infections, immediate death of the fish resulted from asphyxiation.

During its parasitic stage, M. margaritifera increases in size and adjacent lamellae become enclosed within the cyst wall. Approximately 15 lamellae may be fused to the cyst wall of a parasite 250 to 350  $\mu$  in length. Lamellae from the unparasitized side of the gill

filament also commonly fuse to the cyst. The respiratory capacity of these lamellae, except for the outermost one (for example, number 6 in Fig. 13), has essentially been eliminated. Because of the distance involved, it is unlikely that oxygen can diffuse into the others. Also, the physiological dead space in the water flow over the gills, as described by Hughes (1966), increases near the large cysts because they displace lamellae on the filament to which they are attached and on adjacent filaments. This results in an increase in the volume of the nonrespiratory water flow.

Cysts located in the gill arches and rakers occur in the epithelial tissue covering these structures. Since the branchial arteries lie within the connective tissue of the gill arch, blood flow cannot be impeded by the pinching of glochidia. It is also doubtful that these cysts significantly alter the respiratory water flow over the gills.

In conclusion, I believe that the respiratory capacity of the gills is significantly reduced when fish have heavy glochidial infections. Reduction of blood flow in lamellar capillaries and in filamental arteries due to glochidial attachment, and reduction in the number of functional lamellae because of fusion probably represent the major stresses. However, the subtle effect of an increase in the physiological dead space caused by large glochidial cysts must also represent a respiratory stress. Under these conditions the fish would have to pump more water over its gills to maintain an adequate oxygen level

in its blood. If the blood flow has been severely reduced, the fish will probably die of asphyxiation. Murphy (1942) and Matteson (1948) stated that asphyxiation was the principle cause of immediate death in their heavily infected fish.

Apparently in my naturally infected fish, the number of glochidia that had to be acquired over a short period of time to cause immediate death was never attained. Deaths of captive chinook salmon and cutthroat trout were first observed on exposure days 7 and 9, respectively. All of the moribund and the majority of the dead captive fish had fungal infections with their glochidial infections. Under these conditions deaths were likely due to the secondary fungal infections, probably Saprolegnia sp. The invading or exiting glochidia may have provided the portal of entry for the fungi or in some other way weakened the fish making them more susceptible to fungal infection. Murphy (1942) and Matteson (1948) ascribed the delayed deaths of their less heavily infected fish to secondary fungal or bacterial infections.

For reasons previously cited, my steelhead trout and coho salmon probably died from starvation rather than from glochidiosis.

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